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# ANNUAL REVIEW OF MEDICINE:

## Selected Topics in the Clinical Sciences

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## Selected Topics in the Clinical Sciences

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# SOME IMPLICATIONS OF POPULATION CONTROL

❖7175

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## INTRODUCTION

There has been a growing public awareness that unchecked population growth represents one of mankind's most serious threats. The few voices raised over the past decades have swollen to a chorus. The fact that 1974 was designated by the United Nations as World Population Year served to bring the topic to an international forum for open discussion by politicians and policymakers.

As part of the celebration of World Population Year, the World Population Conference, attended by representatives from 137 governments, was held in Bucharest, Rumania, August 19–30, 1974. Responding to this, 450 physicians and population experts from 72 countries participated in an international conference on "The Physician and Population Change," held in Stockholm, September 4–6, 1974. This latter conference was convened by the World Medical Association in cooperation with the World Federation for Medical Education, the International Planned Parenthood Federation, and the World Health Organization.

## THE SITUATION

Berelson (1) states that there are a dozen or more developing countries of small to moderate size in which valid statistics unambiguously document a strong decline in fertility during the 1960s. He writes: "... it is likely that the 1960's marked the beginning of a worldwide fall in the fertility of developing countries—likely but not yet certain." Ravenholt and Chao (2) maintain that these trends are continuing into the 1970s "in many countries, both rich and poor, on every continent."

The optimists can take heart from these remarks, but it is sobering to recall that declines in fertility in most instances will have to be extreme to approach national replacement levels. No nation apparently has achieved replacement; all nations are growing. It probably remains true that we are adding 80 million people globally each year and that the doubling time for the globe's population is in the neighborhood of 32 years. (In the United States we are adding one and a half million people annually with a doubling time of over 100 years.)

The upward spiral in the world's population stems not so much from a change in the fertility rate as from a sharp drop in the death rate. This drop will undoubtedly continue to be seen in more countries as sanitation, vaccination, malaria control, and medicine reach more and more people.

Accordingly, it is almost inevitable that the world's population will grow to between 6.5 billion and 8.5 billion in the next 75 years, with nearly all the growth taking place in the less developed countries (LDCs) (3).

Frejka (4) has calculated that our population would grow to 6.3 billion even if reproductive rates were brought down to replacement levels as early as 1980. Achieving a replacement rate by the year 2000, a more plausible but by no means a certain event, would produce a world population of 8.2 billion by the year 2050, with more than 90% of the additional 4-plus billion in the LDCs.

These figures indicate the phenomenon of momentum which plagues all calculations of population growth. Thus, for example, if the United States came to the instant decision to practice absolute replacement (every girl having a daughter who grew up to have a daughter, every boy having a son who grew up to have a son, etc), our population would increase from the current 211 million to about 300 million before the curve flattened out. There is a lag time produced by the momentum inherent in the structure of the pyramid of age distribution.

Thus, short of a catastrophic rise in death rates, a population increase of major dimensions is in store. The figure of 8 billion would seem to be the minimum, and stabilization may not occur this side of the 10 to 15 billion range. Since 16 billion is a frequently cited figure for the global "carrying capacity," it can be seen that the species *Homo proliferans* is embarked on a path that at best is characterized by brinksmanship.

## THE MEDICAL PROFESSION

What has any of this to do with physicians? Are there not enough other people (politicians, policymakers, lawyers, social workers) to worry about these matters and let the physician continue his comfortable occupation of healing the sick?

There are, however, several reasons why this topic is of special interest to the physician over and above his interest as a concerned citizen. In the first place, since the phenomenon has fundamentally been created by a decline in the death rate, the physician and medicine in general must be recognized as contributing to the creation of the problem. In addition, the weapons to attack the problem (contraception, abortion, and sterilization) are all part of the medical armamentarium. Finally, the relationship between health in general and the practice of family planning and child spacing is now well recognized (5).

At the Stockholm conference this issue was not even debated, and the premise was taken as given that population change was a medical responsibility (6, 7). Dr. Jose Felix Patino, Executive Director of the Panamerican Federation of Associations of Medical Schools, set the tone of the conference in his opening statement that "overpopulation has become the most important factor affecting the health of mankind" (8). The conference ultimately adopted a Strategy for Action (7) which focuses on the four crucial roles in which physicians can effect demographic change and improve the quality of life:

(*a*) as practitioners and family counselors,
(*b*) as teachers and educators,
(*c*) as research scientists,
(*d*) as community leaders.

It might be well to examine briefly the panel reports of the conference which led to the formulation of the Strategy of Action. The first panel examined the role of curative and preventive medicine in influencing population trends. Whereas medical findings and technology helped cause the decline in death rates which has contributed to population growth, the widespread application of this technology received its major impetus from social and economic forces beyond the physicians' control. "Today physicians find themselves in the role of the sorcerer's apprentice, unable to hold back the flood of population growth they helped initiate." The desire to live is instinctive and strong, constituting an irresistible force in combating mortality. The desire to limit procreation, on the other hand, is affected by other forces not yet completely understood. The panel emphasized that, whereas physicians have a key role in developing new technology for the management of fertility, the application of such technology is a legitimate concern of the whole community. Physicians, therefore, must work within the socioeconomic and cultural framework of the community. They should not, however, be passive or silent, but should assume a leadership role.

The second panel focused on the well-proven effects of family planning in substantially reducing health risks for mothers and children and concluded that family planning should become a routine part of patient care everywhere. There is increasing evidence, the panel agreed, that the health benefits of the oral contraceptives are so much greater than the health hazards that this method should be disseminated as widely as possible through every available channel. This could be done, the panel noted, with a minimum of professional supervision through community-based nonmedical facilities. (Actually, of course, all contraceptive methods have a lower death rate than does pregnancy itself; this is clearly true in the United States, but even truer in the LDCs.) The panel decried the tendency to make abortion available only as a luxury for the affluent and contraception available only to those with access to physicians.

A third panel considered the attitudes of physicians toward fertility management and reached the consensus that a new approach is needed. Family planning is more appropriate to an egalitarian, voluntary counselor-client relationship than to the traditional doctor-patient relationship. One Third World participant said: "Doctors should avoid projecting their own attitudes to the patients in the form of intrauterine neocolonialism."

The final panel considered the role of medical education in population change. That such education in demographic realities and family planning procedures is often inadequate was agreed on by all. It is often the case that if healthy and humane control of fertility is to be achieved, it is the medical profession which must now change more than their patients or communities. It was emphasized that additional knowledge without inculcation of the right attitude is worthless.

It was agreed that the problem is too massive to visualize attacking it through the single doctor vis-à-vis a single patient. New family planning workers will be needed and the team approach (without necessarily visualizing a physician as a team member) will be required. Some advocated selling the Pill over the counter without prescriptions on the grounds that the visit to the doctor's office contributed nothing significant that could not be achieved by proper labeling.

## CHINA

World Population Year coincided with (although without a cause-and-effect relationship) the first opportunity for an in-depth examination by Western observers of China's population policy and its implementation. It is curious, although probably not surprising, to find that the country with the world's largest population remained out of sight, if not out of mind, during the past decade of expanding awareness and impassioned debate relating to population growth. But one heard only indirectly and often in contradictory terms of the activities in the People's Republic of China with its more than 800 million people. Now, however, reports (9, 10) are available which indicate that population growth is a matter of official concern at the government level and that the "planning of births" is the official slogan (to be contrasted with our mouthings about "every family's right" and "freedom of choice").

At the time of the Revolution, China accepted the Marxist view that the penalties of population growth were correctable by equitable distribution of wealth, and hence were a by-product of capitalism. But by 1956 the leadership became aware that the rate of population growth was a serious deterrent to economic development, and Chou En-Lai issued a statement endorsing family planning. With the Great Leap Forward, however, public pressure declined, although contraception, abortion, and sterilization continued to be available. Indeed, Peking now insists that the basic policy encouraging planned births was never altered but that birth control simply became inactive.

Beginning in 1962 the Chinese resumed publication of articles encouraging limitation of family size to protect the health of the mother and child. The medical profession was heavily involved at this time with articles in the medical journals and the sponsorship of medical symposia on the subject. Since the Cultural Revolution, China has embarked on an intensive campaign to cut the national birth rate significantly, and the basis has shifted from maternal and child health to national need. "Along with the practice of a planned economy there should also be the planning of births" is one of the current slogans.

Their approach to family planning does not resemble any method to be found in any other country. It is patterned after the numerous other campaigns that have been so successful in China in the past. Orleans says (10),

> In a way perhaps it could be likened to an amalgamation of the spring sanitation campaign which involves the whole community in clean-up activities, and the anti-Confucius/anti-Lin Piao campaign, which involves the whole community in study and discussion activities. Everyone is expected to be involved in the new ideology and the habit of "practicing birth control for the revolution."

It is apparent from reports that all methods of fertility control are available. An oral steroid pill (variously reported to be one third to one quarter the usual dosage in the United States) is made with an annual production sufficient for 20 million women (11). Suction curettage is also available with a highly simplified suction apparatus which is readily available in the countryside. Both vasectomy and bilateral partial salpingectomy are offered and, while specifics are difficult to obtain, there seems to be general agreement that both operations are on the increase.

The outstanding characteristic of the implementation of this policy is its grass roots nature. Enforcement and motivation are the responsibility of the communes and work brigades. "Experience exchange" meetings and discussions which go beyond production quotas and problems, and study topics which relate to family planning are common, and workers in a factory will often announce the number of deliveries they will collectively have during the coming year, while charts are often displayed on hospital walls indicating the number of planned pregnancies permitted for the current year. The approach seems to be effective, although by our standards it involves the loss of a certain amount of privacy and individual decision making.

## CONTRACEPTIVE TECHNOLOGY

The principle of copper-bearing intrauterine devices received FDA approval and one of them (the "7") has been marketed. The addition of copper to the devices increases their effectiveness (almost in a straight-line relationship with the area of copper employed), whereas the expulsion rate seemingly remains a function of shape.

Since the reintroduction of intrauterine devices in 1965, there have been 39 reported deaths in the United States considered attributable to their use. During this same period approximately 8.8 million intrauterine devices have been distributed in the United States. There has been particular concern about the safety of the Dalkon Shield, a concern which stemmed from the fact that 13 deaths from sepsis in pregnancy were reported. In addition there were collective reports of 219 infected pregnancies in which the Dalkon Shield was present. Tatum (12, 13), in some elegant medical sleuthing, reached the conclusion that the multifilament tail with its wick-like action and tendency to split was chiefly responsible for this high infection rate. After a brief time of FDA disapproval, the Shield was reapproved with the proviso that all tails should be monofilament. Whether or not this was the entire explanation for the morbidity and mortality remains to be seen.

Also under the heading of complications and side effects from contraceptive use, a case controlled analysis of the New York State experience with oral contraceptives indicated an increase in limb defects in the offspring of users of oral contraceptives.

Analysis of this data (14) indicated a 7.3% increase among the children of women who conceived while taking the Pill, compared to conceptions among users of oral contraceptives. Since so few pregnancies occur during Pill use, this risk translates into seven defective infants per million users, a sufficiently small public health risk as to have limited policy consequences. Among such consequences will probably be appropriate warnings in labeling of oral contraceptives and a discontinuation of the use of hormonal pregnancy tests.

A considerable amount of investigative work in the realm of applied contraceptive technology is being actively pursued and several methods are currently undergoing clinical testing (15). Unfortunately, a simple enumeration of these, while interesting and perhaps informative, results in rather a "laundry list" presentation.

Prominent among such methods are those which actually represent a new vehicle for delivering the same, already-utilized steroids. Most of these new approaches rest on the interesting and probably valuable properties of silastics which will gradually release, through a container wall, steroids placed within the lumen of that container. This has permitted the development of subdermal implants small enough to be placed through a hypodermic needle and capable of being "worn" many months without local irritation. Appropriate combinations of such implants carrying estrogens and progestins are available for women and estrogen-testosterone combinations are being tested in the male. Another version of this approach is the Silastic vaginal ring, which is filled with steroids that are readily absorbed transvaginally and which can be inserted or removed by the patient in much the same way as a diaphragm, although on the reverse schedule (removed for coitus, worn at all other times). All of these methods show promise and may soon be available, but all, as indicated, simply represent new routes for the delivery of familiar drugs.

Under this same heading, intrauterine devices which carry steroids (largely progestins) in Silastic containers are undergoing clinical testing. Here the objective is not so much to achieve systemic absorption, as with the vaginal rings, as it is to achieve a local effect, rendering the endometrium unsuitable for implantation.

Little progress has been made to date on the development of a new long-term (three to six months) injectable substance. Preparations which provide protection on such a schedule either have undesirable side effects, or remain to have their side effects accurately evaluated. A contraceptive having this particular mode of administration and duration of action is particularly necessary as long as the sword of FDA disapproval hangs over the head of Depo-Provera. In the meantime, Depo-Provera continues to be used, experimentally in selected institutions and extensively abroad—largely in the LDCs—without any accumulating evidence that it is harmful to the human being, even after long periods of use. During the latter part of 1974 it was on the verge of receiving FDA approval when a strong statement from Representative Fountain led officials to reconsider.

Studies continue in many centers in an effort to determine the appropriate place for the prostaglandins in this field. To date one of the greatest areas of promise is in the intrauterine (either intra- or extraamniotic) administration for midtrimester abortion. The various prostaglandins are apparently luteolytic, however, although their use for this purpose requires limited timing in administration and is unpredictable in result. Other nonbiologic chemicals which are luteolytic are being sought, at the moment without too much encouragement as far as human application is concerned.

The objective of such research in applied contraceptive technology is not to achieve a method of greater effectiveness. If efficacy were the principal criterion, one could stop with the development of the oral contraceptives. Rather, the objective which guides such developmental investigation is to achieve a method or methods of such simplicity that they will reach the ill-informed and the ill-motivated. Complicated schedules which involve counting the days of the cycle are beyond the practical understanding of most of the people on the globe. One would gladly give up one or two percentage points of effectiveness if one could reach a million more people through a simple and easily understood approach. Multiple methodologies will be necessary to accommodate different ethnic groups with a variety of cultural heritages.

The very disparity of the groups which must be reached (both nationally and globally) indicates that in addition to scientific research in reproductive biology and technological research in contraceptive development, there must be concentrated study of the sociological variables involved. The determinants of desired family size, the factors influencing acceptability of any particular method—these and many other subjects need to have careful evaluation and study. The population problem is too big and its solution too important to be attacked with inadequate measures. The only alternative to a fall in the birth rate remains a rise in the death rate, not a viable alternative either humanly or professionally.

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# STRESS ULCERATION IN THE ACUTELY ILL

❖7176

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## 1 INTRODUCTION

Stress ulceration may occur acutely in patients who are seriously ill from a wide variety of other systemic conditions. In this review we provide a descriptive definition of this disease, discuss its pathogenesis both in terms of what is known and what is speculative, describe the various clinical situations in which it occurs, and discuss current approaches to diagnosis, prevention, and treatment. Although brevity may be achieved at the price of selective omission, an attempt is made to include important material, even if controversial.

## 2 DEFINITION OF STRESS ULCERATION AND DIFFERENTIATION FROM OTHER TYPES OF ACUTE GASTRODUODENAL BLEEDING

The literature is replete with reports that describe patients with erosive gastritis, hemorrhagic erosive gastritis, stress ulceration, gastric erosions, acute gastroduodenal ulceration, or simply bleeding from the stomach or duodenum in association with other serious illness. Much of the confusion that has existed in the past concerning the entity of acute stress ulceration or hemorrhagic gastritis has been the result of failure to differentiate between patients whose bleeding is related to acute reactivation of a preexisting chronic duodenal or gastric ulcer, and patients whose episode of bleeding is truly due to acute hemorrhagic gastritis. In this review, we exclude those lesions that are more properly classified as chronic active duodenal or gastric ulcers, both of which may obviously be associated with episodes of hemorrhage during other acute illnesses. We make no distinction, however, between the terms *hemorrhagic gastritis* and *acute stress ulceration.* The former term simply defines the background gastric mucosal lesion upon which is sometimes superimposed acute superficial erosions and ulcers. Another point of semantic confusion should be clarified because some authors make much of the distinction between an erosion and an ulcer, presumably because of differences in manifestations or complications. Since acute erosions (ulcers superficial to the muscularis mucosa) may progress to true ulcers into the submucosa after trauma and sepsis, there seems little point in attempting to differentiate these because they are pathogenetically and clinically similar. Extension of the ulcerative process into the submucosal layer where major blood vessels are present is more likely to be associated with major hemorrhage (Figure 1). Although we speak of hemorrhagic gastritis, the anatomical process is primarily ulcerative and only secondarily inflammatory (Figure 1).

Most commonly, acute stress ulceration begins as multiple shallow ulcerations located in the fundic region of the stomach (1) (Figure 2). The submucosal scarring

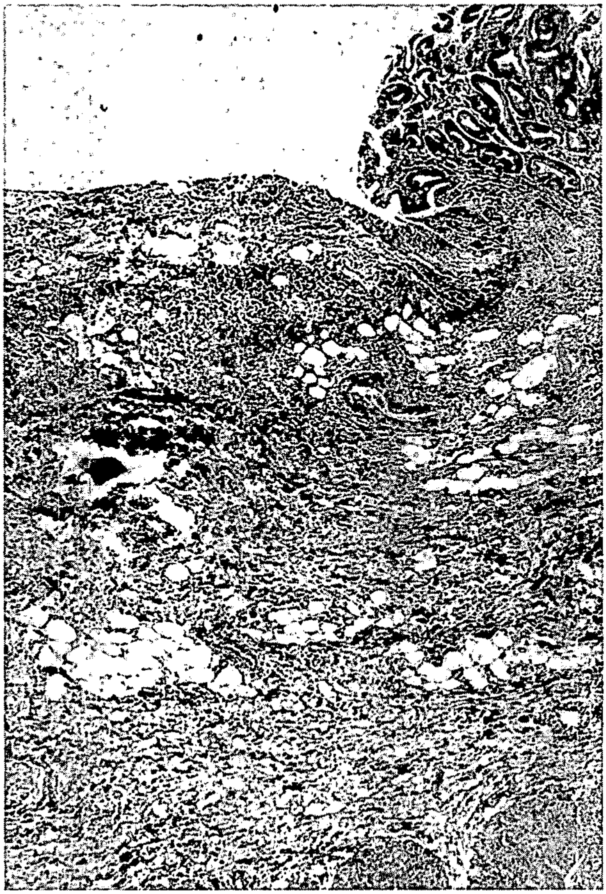

*Figure 1* Photomicrograph of an acute ulcer (1). There is mucosal destruction with extension of the ulcerative process into the submucosa. The submucosa shows pronounced edema and acute inflammation. Intact mucosa is seen in the upper right and muscularis propria is seen in the lower part of the figure. Hematoxylin and eosin stain; original magnification X 30.

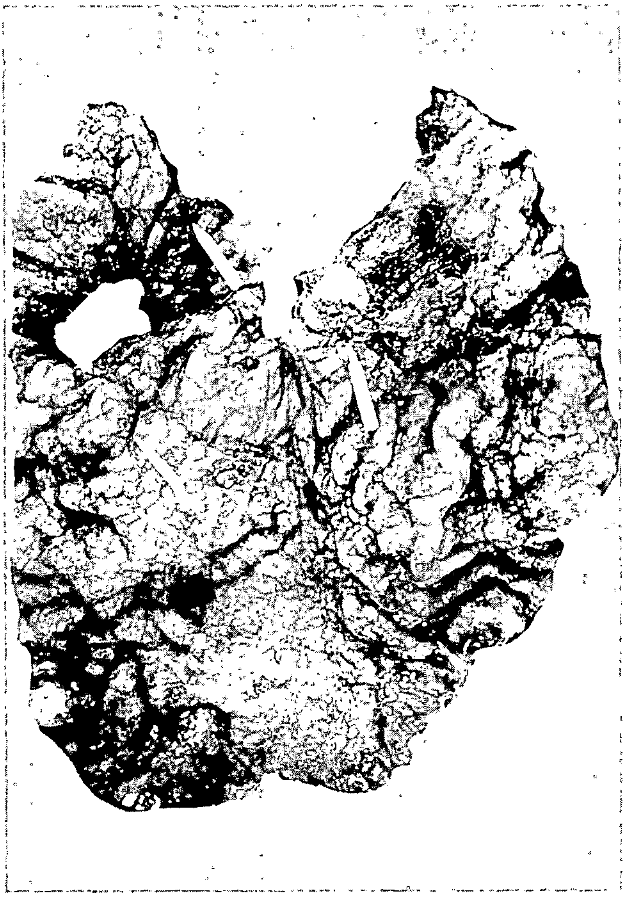

*Figure 2* Gross mucosal appearance of subtotal gastrectomy specimen from a 72-year-old man who had a gangrenous perforated cecum and who bled massively from these ulcerations 6 days after right colectomy (1). The gastrostomy site is seen in the anterior wall. A large acute hemorrhagic ulcer on the posterior wall of the gastric corpus (*upper right*) as well as several smaller ulcers (three indicated by arrows) are present.

which is typical of chronic benign peptic ulcers is completely absent (Figure 1). This is consistent with the fact that these lesions occur within minutes to hours of an acute episode of trauma or other serious illness. Serial fiber-optic endoscopic examinations of the stomach performed within 72 hours of the time of injury in 42 consecutive patients who were severely traumatized showed that multiple gastric erosions located predominantly in the fundic region were found in 100% of the patients examined (2). Within 24 hours of injury, petechiae, and multiple shallow, red-based erosions were observed in the fundic area of the stomach. By 48 hours, the erosions were deeper and frequently were black based with swelling at the margin. In the patients who had progressive gastric mucosal disease, the erosions spread to involve the entire body of the stomach. None of the patients in this study (2) had isolated involvement of the antrum and three patients with antral erosions had extensive disease of the proximal stomach. In certain critically ill patients who had multiple episodes of bleeding, the erosions persisted for as long as three weeks. The sequence of pathological changes in the stomach gradually reversed in those patients who recovered.

Most physicians and surgeons would regard the clinical features observed in the study of Lucas et al (2) as typical of acute stress ulceration. Hemorrhagic gastritis is often unrecognized because early endoscopy is not routinely practiced unless clinically significant bleeding occurs. Minor bleeding from the stomach (indicated by guaiac-positive gastric juice) is frequent in patients whose major clinical problem is severe multi-organ system trauma (3). Massive bleeding from the stomach is fortunately seen in less than 5% of critically ill patients (1). When the primary disease associated with the clinical occurrence of these lesions is successfully treated, mucosal regeneration usually occurs and the integrity of the gastric mucosa is restored (Figure 3).

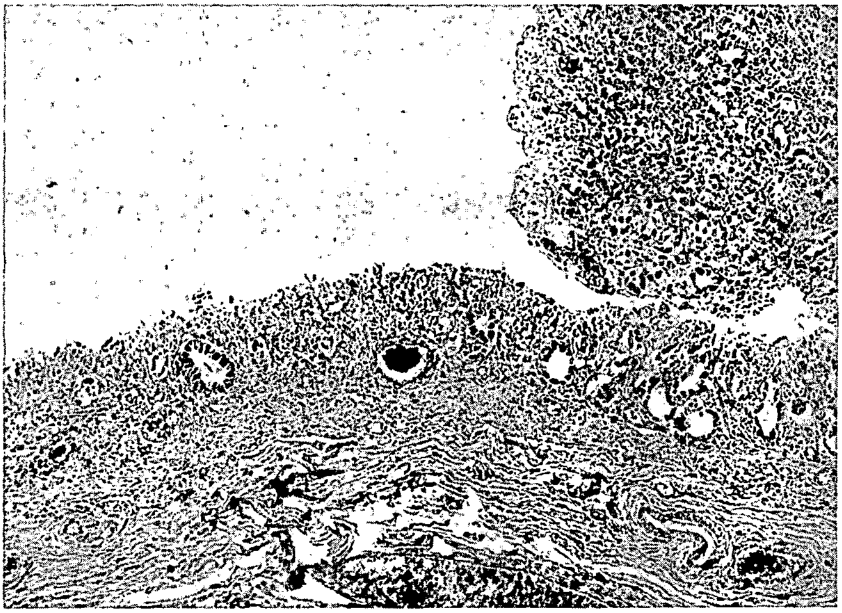

*Figure 3* Photomicrograph of healing ulcer (1). The mucosa is markedly thinned and is composed of regenerating epithelial cells, which are forming irregular, dilated glands and relatively increased stromal elements. Active ulceration is absent. Uninvolved mucosa is seen at the upper right. Hematoxylin and eosin stain; original magnification X 40.

Acute gastroduodenal ulceration usually occurs in four clinical situations, although overlap of these groups is often apparent. Probably the most common situation in which this disease is encountered is in a patient who has had severe trauma. The patient is often in the postoperative period and the added features of hypotension, sepsis, jaundice, and renal and respiratory failure are usually singly or collectively present. Stress ulceration may occur in patients who have had widely varying types of operations or trauma (3–5). Suffice it to say that any patient who develops severe complications after a major surgical procedure may be at risk. The second clinical situation in which acute stress ulceration occurs is after major burns (Curling's ulcers). A third clinical setting in which acute gastric or duodenal ulceration may occur is during or after intracranial disease, trauma, or operations (Cushing's ulcers). The fourth group of patients who develop acute bleeding from the stomach are those patients who have ingested drugs that affect the gastric mucosa. The first two groups should be regarded as examples of true acute stress ulceration in our opinion. They show a number of similarities which are discussed below. The latter two groups are characterized by a number of differences from the first two groups and should probably be regarded as examples of acute gastrointestinal ulceration which may be pathogenetically different from true stress ulceration (6).

## 3 PATHOGENESIS

Interest in the gastric mucosal barrier as an important pathogenetic feature of stress ulceration was stimulated by the investigations of Davenport. Although the existence of a barrier to the diffusion of hydrogen ions from gastric lumen to mucosa was proposed over 40 years ago by Teorell (7), experiments by Davenport beginning in 1964 (8) stimulated new investigation of this important concept in laboratory animals and in normal and diseased man. Teorell (9, 10) suggested that there was a continuous diffusion of hydrochloric acid from the lumen to the mucosa, a process he termed back-diffusion. The amount of hydrogen ion diffusion into the mucosa from the lumen was linearly related to the intraluminal concentration of hydrogen ion. Although Teorell originally proposed a direct exchange of hydrogen ions for sodium ions, Altamirano (11) and others (12) were unable to demonstrate a specific exchange mechanism for these two ions.

It has been suggested that the ability of the stomach to withstand a transmural concentration of hydrogen ions ($H^+$) of $10^6$:1 is related to the presence of a barrier which has thus far not been completely characterized either from a physiological or anatomical standpoint. The known ability of $H^+$ to traverse gastric mucus rapidly relegates the protective role of mucus against $H^+$ to a minor one. Davenport's studies of the barrier largely concern the effects on mucosal permeability of lipid-soluble chemical agents that are injurious to the mucosa. (Figure 4). Bile salts, detergents, urea, aspirin, and ethanol increase the permeability of the gastric mucosa to intraluminal $H^+$, both in the experimental animal (13–17) and in man (18, 19). It is thought that the $H^+$ produce direct injury to the mucosal cells.

Although Davenport suggested that the lipoprotein layer of the plasma membrane of gastric mucosal cells constitutes the barrier and that substances which

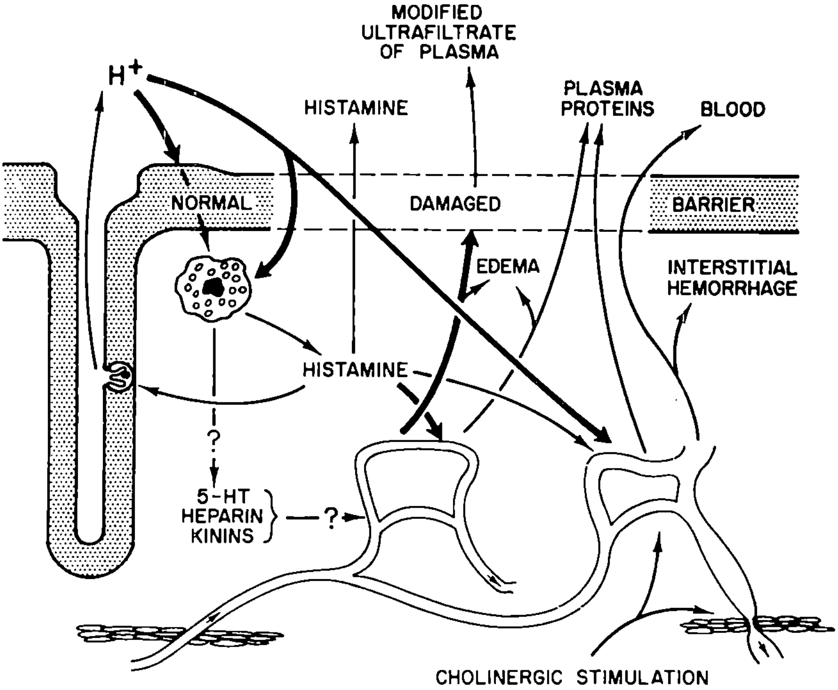

*Figure 4* Schema of events proposed by Davenport leading to fluid production by gastric mucosa that is damaged by a variety of chemical agents (13–17). Secreted acid diffuses slowly through normal gastric mucosa, but diffuses more rapidly after damage. Histamine, heparin, and kinins may be released, which cause further acid stimulation, increase of capillary permeability, and subsequent plasma protein loss into the gastric lumen. Interstitial fluid enters the lumen. More acid enters the mucosa, causing capillary rupture and bleeding, an effect that may be increased by cholinergic stimulation. [From (3a), reproduced with permission.]

increase back-diffusion of $H^+$ cause disruption of the tight junctions between epithelial cells (17), direct anatomic evidence in support of his theory is lacking. Studies of the gastric mucosa in mice showed that the tight junctions between epithelial cells were intact after exposure to aspirin even though, at this same time, marked intracellular injury to mucous neck cells was already apparent (20).

It is possible that the barrier to $H^+$ in the stomach cannot be defined anatomically. The functional state of the mucosal surface and oxyntic cells may play an important role in how much $H^+$ back-diffusion can be tolerated by the mucosal cells before ulceration occurs (21). If vascular perfusion of the gastric mucosa is normal, the alkaline tide created in the oxyntic cell by the generation of $H^+$ secretion may buffer a greater amount of $H^+$ than is possible during mucosal ischemia, when $H^+$ secretion is diminished with consequent reduction in alkaline tide. Evidence to support this contention has been provided by in vitro experiments on frog gastric mucosa in which artificially induced $H^+$ back-diffusion has been shown to be accentuated by inhibition of spontaneous acid secretion with burimamide, a histamine ($H_2$) receptor antagonist (22). Whereas previous studies of gastric mucosal permeability have focused on the absolute amount of $H^+$ that leaves the gastric lumen to enter the mucosa, redefinition of the barrier may require an assessment of the amount of $H^+$ that can be buffered by the mucosa at any one time. Normal mucosal blood flow may completely clear back-diffusing $H^+$ and thereby protect the mucosa from its damaging effects, but when $H^+$ accumulate in the mucosa, ulceration may occur.

Acute stress ulceration in man is usually preceded by an episode of shock. A decrease in gastric mucosal blood flow is an important underlying mechanism for the development of experimentally induced acute gastric ulceration in a wide variety of animal preparations (23–27). Whether or not a diminution of mucosal blood flow can be the sole cause of acute ulceration remains a debatable issue. Although one study of hemorrhagic shock in rabbits showed significant back-diffusion of $H^+$ and acute ulceration of the stomach with pylorus and cardia ligated 3 hours after the shock period (26), another study in dogs subjected to hemorrhagic shock demonstrated ulceration of the stomach only in those animals in which the gastric mucosa was exposed to bile (28). A study of hemorrhagic shock in the dog showed that acute gastric ulceration was invariably preceded by reflux of duodenal content into the stomach (23). In these latter experiments, inhibition of the trypsin in the chyme prevented the development of gastric ulceration and the associated intestinal lesions. Pyloric occlusion, which excluded duodenal chyme from the stomach also significantly reduced the incidence and severity of the gastric ulcerations. These experiments suggest that in hemorrhagic shock, the gastric mucosal barrier may be disrupted by reflux of duodenal content into the stomach as well as by ischemia alone. Reflux may be an important factor in many critically ill postoperative patients with adynamic ileus in whom stress ulceration commonly occurs.

Experiments performed in canine vagally denervated fundic pouches have shown that gastric mucosal blood flow determined by the aminopyrine clearance technique is decreased by acid solutions containing 10 or 40 mM concentrations of taurocholate or a 20 mM concentration of aspirin (29). This finding suggests that these agents may be damaging to the gastric mucosa not only by causing back-diffusion of $H^+$, but also by producing mucosal ischemia.

Although back-diffusion of acid was not detected after hemorrhagic shock by the experimental technique employed by Moody & Aldrete (30), it is significant that ulcers developed in these experiments only when acid was present. Thus, it appears that it is not the absolute amount of acid entering the mucosa that is an important pathogenetic factor in the development of the ulceration, but rather back-diffusion of acid into a mucosa incapable of disposing of it. Hypersecretion of acid is not a consistent finding in patients who develop stress ulceration (5, 26, 31). Although it is known that increased back-diffusion of $H^+$ following instillation of an acid solution (100 mN HCl) occurs when canine gastric mucosa has been previously exposed to a solution of bile salts at a pH of 8.0, back-diffusion of $H^+$ is quantitatively less at a pH of 8.0 than when the bile salts are adjusted to pH $= 2.0$ (32). Even a small amount of acid appears to be capable of producing these lesions in the presence of ischemia. The absolute amount of back-diffusion of $H^+$, upon which almost everyone has focused, may therefore be unimportant. Although anoxia by itself does not produce a large increase in $H^+$ back-diffusion in experimental in vitro preparations of gastric mucosa (33), the combined effects of ischemia and other damaging agents regularly do produce ulceration (34). Large quantities of pepsin are released by many agents which produce disruption of the gastric mucosal barrier (35). In the presence of ischemia and acid, release of pepsin may be an additional ulcerative factor in patients who are at risk of developing hemorrhagic gastritis (36).

A severe deficit in gastric mucosal energy metabolism has been shown after the induction of hemorrhagic shock in the rat (37). Other experiments in the rabbit indicate that the breakdown of high energy phosphates (reduction of adenosine triphosphate levels in the gastric mucosa) is significantly greater in fundic mucosa compared to antral mucosa (38). These experiments provide a possible clue to why stress ulcers may be more common in the fundic region (1, 2), and support further the contention that the amount of acid in the mucosa in relation to the state of mucosal energy production may be a critical determinant of whether or not ulceration will occur.

Many drugs are known to produce increased permeability of the gastric mucosa to acid. Prominent among those that have been implicated in the development of clinically significant hemorrhagic gastritis are aspirin, ethanol, indomethacin, and steroid preparations. Aspirin, ethanol, and indomethacin have been shown to produce an increased back-diffusion of $H^+$ in animals (15–17, 39, 40) and in normal man (18). While prednisolone by itself causes no perceptible effect on canine gastric mucosal permeability, this drug has been shown to enhance back-diffusion of $H^+$ caused by bile salts, aspirin, and acetic acid (41, 42). However, ferrous sulfate, an agent that occasionally causes clinically significant gastric irritation, and acetaminophen, an antipyretic agent often substituted for aspirin, do not produce an increase of gastric mucosal permeability in normal human subjects (43).

## 4 STRESS ULCERATION AND OTHER TYPES OF ACUTE GASTRODUODENAL ULCERATION IN THE ACUTELY ILL

### *4.1 Stress Ulceration in Association with Trauma, Sepsis, and Postoperative Complications*

Lesions typical of true acute stress ulceration in this seriously ill group of patients are described in detail in Section 2. Sepsis, hypotension, jaundice, and renal and respiratory failure are the typical clinical features observed in the group of patients who are at high risk of developing this disease (1, 26, 36). Increased permeability of the gastric mucosa to $H^+$ was correlated with a higher incidence of these clinical complications in two studies of critically ill patients (26, 36). Gastric mucosal ischemia, bile reflux into the stomach, reduction of the gastric mucosal cellular content of ATP, increased back-diffusion of $H^+$, and the ability of the gastric mucosa to tolerate the acid load are probably all important determinants of the development of ulceration in this group of patients. It is probable that lesions which are true stress ulcerations may also occur in the duodenum in patients who are acutely ill. The mechanisms responsible for acute ulceration in this location have not yet been clearly identified.

### *4.2 Stress Ulceration in Association with Burns (Curling's Ulcers)*

Although the duodenum was the source of massive bleeding in the 12 children and young adults originally described by Curling in 1842 (44), more recent reports by Sevitt (45) and O'Neill and co-workers (46) indicate that multiple superficial gastric ulcerations are a more common source of bleeding in these patients. A recent endoscopic evaluation of 32 adult patients with burns (47) indicated that multiple gastric erosions were present in 25 of 29 (86%) with large burns (mean burn size = 56% of the body surface area), 74% of which were evident within 72 hours after the burn. Patients with lesser burns were found to have normal gastroduodenal mucosa. Seven patients developed a gastric ulcer (22%), each of which was located in an area of intense superficial mucosal disease. The earliest detectable gastric ulcer occurred 96 hours after burning. Nine patients had a duodenal ulcer and all but one had concomitant gastric disease. With the exception of the increased occurrence of duodenal lesions in the report of Czaja et al (47), these findings are remarkably similar to those of Lucas and co-workers (2), who also found that mucosal lesions in severely traumatized patients were most commonly located in the fundus and body of the stomach. Both studies suggest that mucosal ischemia is the initiating event and that ulceration occurring early after burns may be similar pathogenetically to ulcers associated with trauma and sepsis. Of further interest in the report of Czaja et al (47) was the finding that patients whose clinical course deteriorated with the onset of sepsis, hypotension, or respiratory insufficiency had progression of the mucosal disease.

An evaluation by the same group (48) of the gastric mucosal barrier in thermally injured patients indicated that 8 of 18 patients with major burns (mean burn size = 57%) had disruption of the gastric mucosal barrier, and 7 of these 8 patients (87.5%) who showed increased mucosal permeability within 72 hours after burning developed gastric bleeding, perforation, or endoscopic progression of mucosal disease. This report suggests that although increased gastric mucosal permeability may not be causally related to the development of stress ulceration after burns, this factor may be an important contributor to the progression of mucosal ulceration.

The observations on burned patients from the Brooke Army Burn Unit (47, 48) indicate that the mechanism of acute ulceration that occurs within 72 hours following burns may be similar to that encountered in patients after major trauma and sepsis. Whether the apparently higher incidence of perforation (1 gastric and 1 duodenal) observed in patients with major burns indicates subtle differences in pathogenesis remains to be determined.

### *4.3 Acute Gastroduodenal Ulceration in Association with Central Nervous System Disease (Cushing's Ulcers)*

Major differences exist between patients who develop acute stress ulceration after trauma or after the development of serious complications, and those patients who develop acute gastroduodenal ulceration in association with central nervous system disease.

Acute gastrointestinal ulceration occurring after intracranial trauma or operations often consists of more deeply penetrating lesions than those occurring in patients with multiple postoperative complications. These lesions may involve the full thickness of the esophagus, stomach, or duodenum, and perforation is more commonly seen in this group of patients (49). A recent comparative study (36) of gastric mucosal permeability in a group of 13 patients with general surgical problems and 9 patients with neurosurgical disease confirmed an earlier report (26) that gastric mucosal permeability was increased in both groups of patients when multiple complications were present. The neurosurgical patients had greatly increased gastric acid secretion and pepsin secretion in comparison with the sickest group of general surgical patients. A positive relationship between increased gastric muscosal permeability and bleeding (gastric aspirate greater than 2+ guaiac for 3 consecutive hours in the 12 hours before study) was strongly supported by this study.

A study of serum gastrin levels in patients injured in Vietnam showed that patients with central nervous system injury have significantly higher levels of serum gastrin than those without injury of the central nervous system (50), although there was no apparent relationship between the levels of serum gastrin and the subsequent development of stress ulceration in these patients. It is possible that the higher levels of serum gastrin found in patients with central nervous system injury are related to increased vagal activity. This suggestion is supported by several studies of patients with head injury, which show elevated gastric acid secretion (36, 51) and elevated concentrations of acid and pepsin in the gastric juice (52).

### *4.4 Acute Bleeding from the Stomach in Association with Drug Ingestion*

The clinical course of drug-induced gastritis is usually less severe than that seen in sick postoperative patients who develop acute gastroduodenal ulceration, and withdrawal of the offending agent frequently results in healing of the mucosal lesions and cessation of bleeding. Reduction of the $H^+$ concentration in the stomach by alkalinization of the stomach with antacids may be helpful in therapy (see Section 6).

## 5 DIAGNOSIS OF STRESS ULCERATION

The most common clinical manifestation of stress ulceration is bleeding. Because the earliest anatomic lesion is superficial mucosal ulceration, attempts to make a specific diagnosis by selective angiography or barium studies are frequently unrewarding. Barium studies also prevent the use of intraarterial infusion of vasopressin for treatment of bleeding since visualization of the arterial tree is obscured by the barium. Early fiber-optic endoscopy has proven to be the most useful diagnostic tool (53, 54). The success rate of gastroscopic diagnosis is higher when the examination is performed within the first 24 hours of hematemesis (53). Endoscopy may be performed in critically ill patients receiving controlled ventilation or hemodialysis directly in the intensive care unit, another advantage of this diagnostic technique.

## 6 PREVENTION OF STRESS ULCERATION

Although anecdotal reports support the contention that antacids may protect against the development of stress ulceration, confirmatory proof of this form of prophylaxis from a properly conducted randomized trial is lacking. Nevertheless, experimental evidence indicates clearly that hemorrhagic gastritis and stress ulcers do not form in the absence of acid (26, 30). It seems reasonable, therefore, to adopt a policy of aggressive antacid prophylaxis in patients who are at high risk of developing this complication. The regimen currently used in our surgical intensive care unit is to titrate the gastric content to a pH greater than 3.5 by instilling 30 to 60 ml of antacid into the stomach every hour through a nasogastric tube. The tube is clamped for 45 minutes and aspirated for 15 minutes. If the pH of the aspirated sample is less than 3.5, twice the volume of antacid is added on the subsequent hour until the pH is greater than 3.5. Simple pH-sensitive paper is used to determine the hourly pH. Antacid prophylaxis has the additional theoretical advantage of inhibiting the proteolytic enzyme pepsin, since the activity of this enzyme is almost completely abolished at a pH of 5 (55). By using this approach, we have gained the impression that the incidence of stress ulceration in the susceptible population has decreased, although, as mentioned above, data from a controlled trial are not yet available to give unequivocal endorsement to this regimen.

Carbenoxolone sodium, the disodium salt of glycyrrhetinic acid, an extract of licorice root, increases the life span of gastric mucosal epithelial cells in experimental animals (56), and has been demonstrated to have a beneficial effect on the healing of gastric ulcers in several well-controlled trials in Britain (57–59). However, this agent has been recently shown to have no effect on reducing gastric mucosal permeability to taurocholate-induced back-diffusion of $H^+$ in a canine model or to ethanol-induced increases of gastric mucosal permeability in normal human subjects (60). In addition, carbenoxolone has potent mineralocorticoid side effects that appear to limit further its prophylactic application to patients who are likely to develop stress ulcers (60).

A number of investigators have postulated that the reflux of bile into the stomach plays a role in the etiology of gastric ulcer and stress ulceration in humans (28, 34, 61, 62). Cholestyramine, a quaternary ammonium anionic exchange resin with a strong affinity for bile salts, has been recently shown to decrease significantly $H^+$ back-diffusion in a canine hemorrhagic shock model of bile-induced mucosal ulceration (63). None of the animals in the cholestyramine-treated group developed mucosal ulceration. No clinical trials of this agent in patients who are at high risk of developing stress ulceration are yet available to assess accurately its prophylatic value.

## 7 TREATMENT OF STRESS ULCERATION

Evidence of early stress ulceration (frankly bloody or guaiac-positive gastric juice) in susceptible patients is probably best treated by aggressive antacid therapy as outlined above. Recently, selective intraarterial infusion of vasopressin has been advocated as a method of treatment of patients who are bleeding massively from stress ulceration (64, 65). Although patients occasionally appear to stop bleeding with this therapy, recurrent bleeding is not uncommon. The only available prospective randomized clinical trial of intraarterial vasopressin in the treatment of patients with upper gastrointestinal hemorrhage (66) showed that vasopressin therapy was more successful ($p < 0.05$) in controlling hemorrhage of nonvariceal origin than conventional therapy, although mortality rate was not improved in the vasopressin-treated patients. Specific data on the effect of this therapy on bleeding from stress ulceration were not provided in this report. It is not possible, therefore, to state with certainty how effective this form of therapy is for stress ulceration.

Operative treatment of stress ulceration is also controversial. Although pyloroplasty and vagotomy has been the most commonly performed operative procedure, the incidence of recurrent hemorrhage in a collected series of patients is approximately 40% (5). As in the previously described methods of treatment, no controlled studies are available to clarify the issue. Our preference for operative treatment is a generous gastric resection with vagotomy, especially for those patients who develop bleeding from stress ulceration in association with trauma, sepsis, jaundice, and respiratory and renal failure. Vagotomy and pyloroplasty may be adequate treatment for patients whose gastric hemorrhage is related primarily to drug ingestion, although even in these patients it is impossible to give unequivocal endorsement to a single operative procedure. Without doubt, the aim should be to prevent the development of these frequently fatal lesions by the approaches already outlined.

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# INFECTIOUS PARTICLES IN HEPATITIS

❖7177

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## INTRODUCTION

The classical approach to a virus disease is to attempt to isolate the agent in a cell culture system, and subsequently to study the morphology and serology of the virus. Advances in the electron microscopy and serology of viruses have meant that this order is now sometimes reversed, i.e. the virus particle (virion) is first characterized, and only subsequently is the virus isolated, i.e. cultured, in the laboratory. This has resulted partly from the high resolution obtainable with negatively stained preparations and partly from the combination of serology with electron microscopy (1) known as immune electron microscopy. In no field of medical virology has this reversal of the classical approach been more evident than in viral hepatitis. Most cases of acute hepatitis in man are not caused by any of the well-known and clearly characterized viruses, or by other agents such as leptospires, but by filterable agents which have not yet been isolated. It is generally agreed that there are at least two of these (2): hepatitis A and B (see Table 1). The latter can be identified in patients and carriers by finding the hepatitis B antigen (HBAg), formerly known as Australia antigen, in the blood, either serologically or by electron microscopy. Hepatitis A has no corresponding antigen in the blood, but particles have been described in the feces of patients and of experimentally infected human subjects. Conventional small laboratory animals have not so far been infected, but hepatitis A has been transmitted to the marmoset, and hepatitis B to the chimpanzee. The position, at the time of writing, is that hepatitis B boasts a DNA-containing particle (3, 4) known as the Dane particle, which is linked with certainty to the clinical illness and bears all the hallmarks of a conventional DNA virus, although it does not share its precise morphology with any at present known. For hepatitis A, on the other hand, several workers have described a particle identical in morphology with the enteroviruses, but which has not been implicated in the illness with such certainty as has hepatitis B.

Table 1 Human viral hepatitis A and B

| | Hepatitis A | Hepatitis B |
|---|---|---|
| Synonym | infectious hepatitis | serum hepatitis |
| HB antigen in serum | negative | positive |
| Epidemiology | fecal-oral spread<br>food- or water-borne outbreaks | close physical contact or transfer of blood and blood products |
| Incubation period | 3-5 weeks<br>(mean 28 days) | more variable and generally longer<br>(~ 60-180 days) |
| Presumed infective particle | approximately 27 nm, enterovirus-like<br>RNA (probably)<br>cytoplasmic | 44 nm—double-shelled virion (Dane particle)<br>DNA<br>nuclear<br>other forms found |
| Found mostly in | feces | serum |
| Corresponding Krugman and Giles' agent | MS1 | MS2 |

## HEPATITIS A

There are now various descriptions of a particle 27 nm in diameter, in connection with hepatitis A (5–7). It is identical in appearance to the virion of the enterovirus group. This particle, found in the feces, has been correlated with the natural disease and with the disease as conferred experimentally on human subjects. Material containing such particles has caused hepatitis in the marmoset, and material from infected marmosets has been used as an antigen for serological studies in man. This particle has also been linked antigenically with the MS1 agent of Krugman et al (8), which is one of the two distinct types of viral hepatitis observed by them. MS1 hepatitis is HBAg negative, and the other (MS2) is HBAg positive and corresponds to hepatitis B. Reports of exchange of sera between various groups working on hepatitis A, and reference to MS1 antiserum as a fixed point, have given reason to believe that all the workers who have described this particle are dealing with the same agent, and that it in turn is the agent of MS1 viral hepatitis.

### *Transmission to Marmosets and the Correlation with Human Infection*

It appears that hepatitis A was first transmitted to the marmoset by Deinhardt et al (9). This has been intensively followed up by Provost, Hilleman, and others. A pool of serum taken in the acute phase from a Costa Rican child with hepatitis A was inoculated by Provost et al (10) into marmosets (*Saguinus mystax*). This caused a hepatitis which was transmissible to further marmosets and could be neutralized by convalescent sera from human cases of hepatitis A. This neutralization served as a serological test for the human disease, but not one which was readily usable because of the shortage of marmosets. The liver of infected marmosets contained particles of size 27 nm (as seen by negative staining) that, in sections of liver cells, were localized in the cytoplasm (11). These particles were also present in the serum of marmosets in the acute phase of the disease. In thin sections of liver both full and empty particles could be seen, as well as groups of particles in cytoplasmic vesicles. It proved possible to use this material as an antigen for three further serological tests: immune electron microscopy, complement fixation (CF) (12), and the immune adherence reaction (13). All three, unlike the neutralization reaction, obviate the use of the marmoset, or indeed of any system in which viral replication occurs. In addition, the marmoset material (infected serum) has been used for a study of the physical properties of the particle, and, because the marmoset can be used as an experimental animal, the effect of various agents on the infectivity can be examined. Unpurified material from marmoset serum is incompletely inactivated in 1 hr at 60°C, but is destroyed in 5 min at 100°C. It is inactivated by ultraviolet irradiation and by formalin (1:1000 for 3 days at 37°C). It is stable at pH values low enough to inactivate rhinoviruses. Morphological considerations make it highly likely that this virus would be an RNA-containing particle, and preliminary fluorescent staining with acridine orange suggests that this is so. Immune electron microscopy of particles, from serum or from liver homogenates, showed specific attachment of antibody from convalescent human hepatitis A serum.

The use of marmoset liver containing this particle, which has become known as CR326, as a CF antigen has shown the development of CF antibody in human patients parallel to the development of neutralizing antibody. It is of interest that the serum becomes anticomplementary as antibody develops (shortly after the onset of the clinical illness), suggesting the formation of circulating immune complexes. One patient developed antibody, which persisted, against normal liver. A patient with HB-antigenemia was shown to have had hepatitis A, rather than B, as judged by a rise in CF antibody to the marmoset hepatitis A antigen. Krugman et al (14) studied the sera of 20 patients with clinical hepatitis A (MS1 type) by the CF test and also by the immune adherence (IA) test. Both tests showed that the agent concerned was antigenically the same as MS1, and that CF and IA antibodies developed during the course of illness. The latter test was found to be more sensitive and more specific than the CF test, and IA antibody levels persisted at high titers in some patients for 5–10 years after their illness.

This work has therefore provided a feasible, if not ideal, experimental animal. It has allowed the examination of the morphology and physical and chemical properties of at least one agent causing acute hepatitis in man, but which is definitely not hepatitis B virus. It has provided a means of preparing CF and IA antigen, and the provision of antigens for these serological tests has in turn made possible the study of the formation of antibody during the clinical illness and has opened the door to epidemiological studies on hepatitis A.

### *Electron Microscope Studies on Natural and Artificial Human Infections*

Direct examination by Feinstone et al (5) of negatively stained preparations of fecal extracts from subjects artificially infected with MS1 virus allowed visualization of a 27 nm particle, morphologically like an enterovirus. These particles reacted, by immune electron microscopy, with convalescent sera from experimentally infected subjects and from natural cases of hepatitis A. The time pattern of fecal shedding was studied by Dienstag et al (15) who found, in a detailed study of two patients, that the particles appeared in the feces before the jaundice, but did not persist after the peak of transaminase levels had been reached.

Apparently identical particles, from natural outbreaks, have been described by Locarnini et al (7) in Australia. These were serologically identical to the particles of Feinstone et al (5). The size (27 nm) and morphology are the same as the particles of the CR326 agent transmitted to marmosets by Provost et al, and they react with convalescent sera from the same outbreaks and also with sera from MS1 patients. Similar particles, also reacting with sera from MS1 patients, were reported in a natural outbreak in Phoenix, Arizona by Gravelle et al (6).

Hepatitis A in Belfast has been studied by Almeida et al (16). In a combined study by gel-diffusion and electron microscopy, they found seroconversion to various fecal antigens, as detected by the gel-diffusion technique. In fecal extracts a number of different particles were found by electron microscopy, and also a varied range of sizes of small spherical particles, including one of 27 nm, to all of which seroconversion occurred. Examination by these workers of material from one of Feinstone et al's patients confirmed the presence of 27 nm particles (Figure 1*a*), but showed also particles of 22 nm and 30 nm. All three of these particles reacted with convalescent sera from the Belfast patients (17). This fecal material caused hepatitis in two out of six marmosets, and hence presumably contained hepatitis A virus. The outcome of these apparently confusing findings is that the significance of antibody rises in acute liver disease has come under scrutiny. When the liver disorder is caused by an acute infection it is reasonable to expect an antibody rise to the infectious agent, but it is also the case that nonspecific antibodies to various antigens occur also (18). This would explain the CF antibody rise to normal liver reported in one case by Provost et al (12) and probably the otherwise somewhat enigmatic rises to cytomegalovirus and Epstein-Barr virus in post-transfusion hepatitis recorded by Purcell et al (19).

In spite of the uncertainty aroused by the undoubted occurrence of nonspecific antibody reactions, it would appear, taking all the evidence together, that the 27 nm particle described in feces by various authors, and transmitted to marmosets by Provost et al (10), is firmly in front as a runner in the hepatitis A stakes, even if it has yet to pass the winning post. However, this is not to say that the 27 nm enterovirus-like particle is the only virus-causing HBAg-negative acute viral hepatitis in man. There may be other serotypes of similar morphology, and there may be other still unrecognized viruses, possibly including some of the particles described by Almeida et al (16), to which seroconversion occurs. The properties of some of these, and other relevant particles, are set out in Table 2. These supposed agents may be particularly common in the blood of commercial blood donors. Be that as it may, the 27 nm particle of MS1 antigenicity is in a strong position. The fact that there is now available for this strong candidate an experimental animal, several serological reactions, definitive information on its morphology and physical properties, and even tentative chemical data on the nucleic acid type, bears witness to how much modern virological techniques can avail, even when a laboratory system of viral

**Table 2** Particles found in viral hepatitis, viral gastroenteritis and some other human infections

| Virus | Size (nm) | Nucleic acid[a] | Buoyant density (CsCl) | Morphology | Clinical significance in man |
|---|---|---|---|---|---|
| Hepatitis B (Dane particle) | 44 (with 27 nm core) | DNA (D) | 1.25 | double-shelled | viral hepatitis B |
| Hepatitis A (CR326) | 27 | ? RNA (S) | 1.34 | enterovirus-like | viral hepatitis A |
| Norwalk virus | 27 | ? RNA (S) | 1.38–1.41 | enterovirus-like | gastroenteritis |
| Rotavirus | 64–66 | ? RNA (D) | — | "reovirus-like", with radiating capsomeres | gastroenteritis in infants, also calf, mouse, etc |
| Coronavirus | 80–160 | RNA (S) | 1.19 | coronavirus structure | gastroenteritis in pigs |
| Enterovirus (poliovirus, Coxsackie virus, ECHOvirus) | 27 | RNA (S) | 1.34 | enterovirus (isometric) | meningitis, poliomyelitis, myocarditis, epidemic myalgia, etc |
| Rhinovirus | 27 | RNA (S) | 1.38–1.41 | rhinovirus (isometric) | respiratory infections |
| Parvovirus | 22 | DNA (S) | 1.38–1.45 | parvovirus (small, isometric) | uncertain |

[a] S = single-stranded; D = double-stranded.

growth is not available. It also makes it probable that the way to such a system for hepatitis A may soon be opened up.

## *Grounds for Associating the 27 nm Particle with Hepatitis A*

The principal finding, common to all the investigations conducted, is that the stools of patients with clinically typical hepatitis A contain particles of a consistent diameter (27 nm) which morphologically resemble enteroviruses. Other particles are found, but the 27 nm is the only particle consistently and ubiquitously associated with cases of clinically and epidemiologically typical hepatitis A. The evidence that this particle is the infectious particle of hepatitis A is as follows:

1. The particle is found in the stools of patients with clinical hepatitis A, but not of patients with serological hepatitis B.
2. The time pattern of shedding in the stools corresponds to the peak of the biochemical changes and of the clinical hepatitis.
3. These particles react, as observed by immune electron microscopy, with convalescent sera from cases of clinical hepatitis A, but not with early sera. The change from no reaction to a positive reaction, although not strictly a quantitative assessment, may be taken to indicate the production, concurrently with the illness, of antibody specific for this particle. This has been correlated with the development of neutralizing, CF, and IA antibodies in the sera of human cases.
4. Material, both serum and feces, from cases of clinical hepatitis A has reproduced the condition in marmosets, and particles resembling these have been found in the serum and liver of marmosets. These in turn can be used as antigens for the

investigation of human sera. It must be admitted that the argument involved is, to some extent, a circular one, but in each of several studies the particles have been serologically correlated both with patients under study and with the MS1 agent of Krugman & Giles (20).

5. The transmission of hepatitis to a chimpanzee, using human hepatitis A material as inoculum, has been reported by Thornton et al (20a). This animal showed an elevation of serum enzymes between 21 and 42 days after inoculation. Particles serologically specific for hepatitis A were found in the feces from 9–28 days, with a maximum at day 19, i.e. before the rise in serum enzymes. Although these particles showed some variation in size (24–29 nm), this does not exclude their identity with those particles reported as 27 nm by other workers, and indeed, perusal of various published electron micrographs (e.g. refs. 5, 7, 11) suggests that the particles specifically identified by immune electron microscopy do in fact show an appreciable spread on either side of 27 nm, "unlike the majority of small cubic viruses" (20a). The presence of a coating of antibody does in any case make very precise measurement difficult, and, even if uniform, the size may prove to be a little smaller, or, more probably, greater than 27 nm, e.g. 28–29 nm. Whatever the precise size of the particle, production of hepatitis in the chimpanzee, associated with excretion of these particles, reinforces the evidence from the marmoset that the particles concerned are the agent of human hepatitis A.

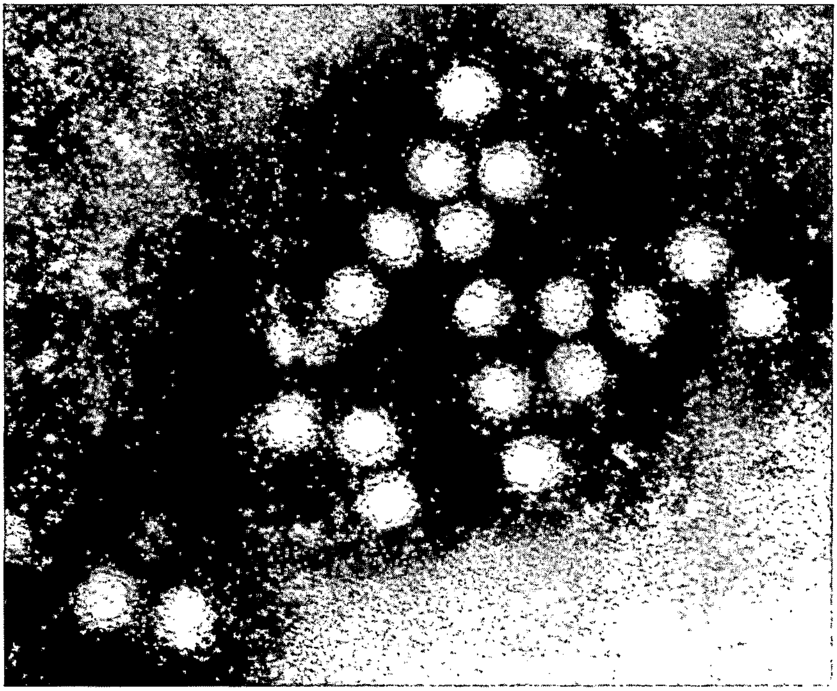
### *Relation to Gastroenteritis Viruses in Man and Other Vertebrates*

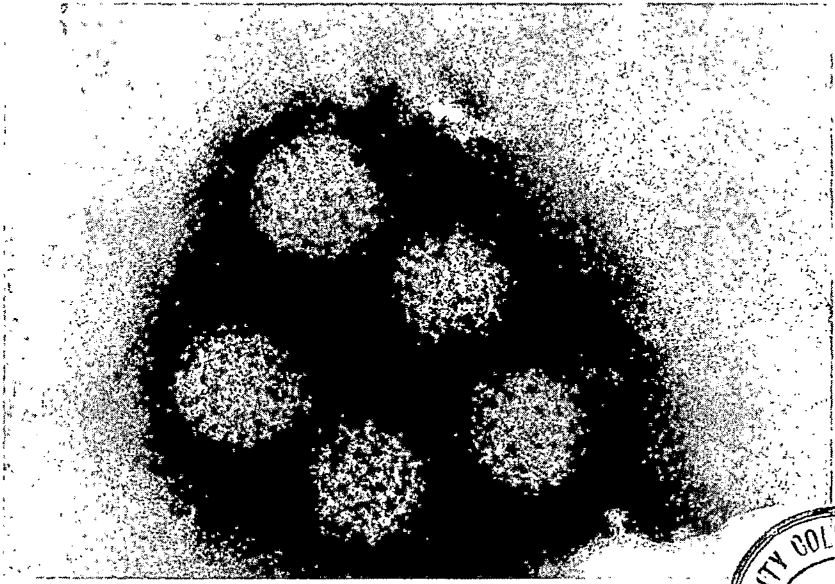
The picture has been complicated, if not actually confused, by the simultaneous search for gastroenteritis viruses. The Norwalk virus, a 27 nm particle under suspicion as a cause of human gastroenteritis, was described in 1972 (21). It is antigenically unrelated to MS1 hepatitis A virus. A new and morphologically quite different group of viruses (the rotaviruses) has been implicated in human and animal gastroenteritis (Figure 1*b*) and may be the principal cause of infantile gastroenteritis (22–24). Outbreaks of gastroenteritis in piglets are caused by a well-characterized coronavirus (transmissible gastroenteritis of pigs) and it seems likely that there may be a counterpart in human disease. Probably representatives of other groups will be implicated also, but, with the exception of Norwalk virus, are unlikely to be sources of confusion.

### *The Role of the Parvoviruses*

Parvoviruses are small (22 nm) isometric viruses with single-stranded DNA and a high buoyant density (1.41) in CsCl. They have been involved as causes of enteritis in the cat and the mink. [The high buoyant density of Norwalk virus led Kapikian et al (25) to suggest that this was a parvovirus also, but the particle size (27 nm)

*Figure 1* *a* Particles (size 27 nm) from feces of patient with acute HBAg-negative viral hepatitis. These have been aggregated with chimpanzee anti-HA serum, and antibody can be seen adhering to the particles. X 275,000. Negatively stained with phosphotungstic acid. *b* Rotavirus particles (65 nm) from feces of calf suffering from gastroenteritis. X 275,000. Negatively stained with phosphotungstic acid. Electron micrographs by June D. Almeida.





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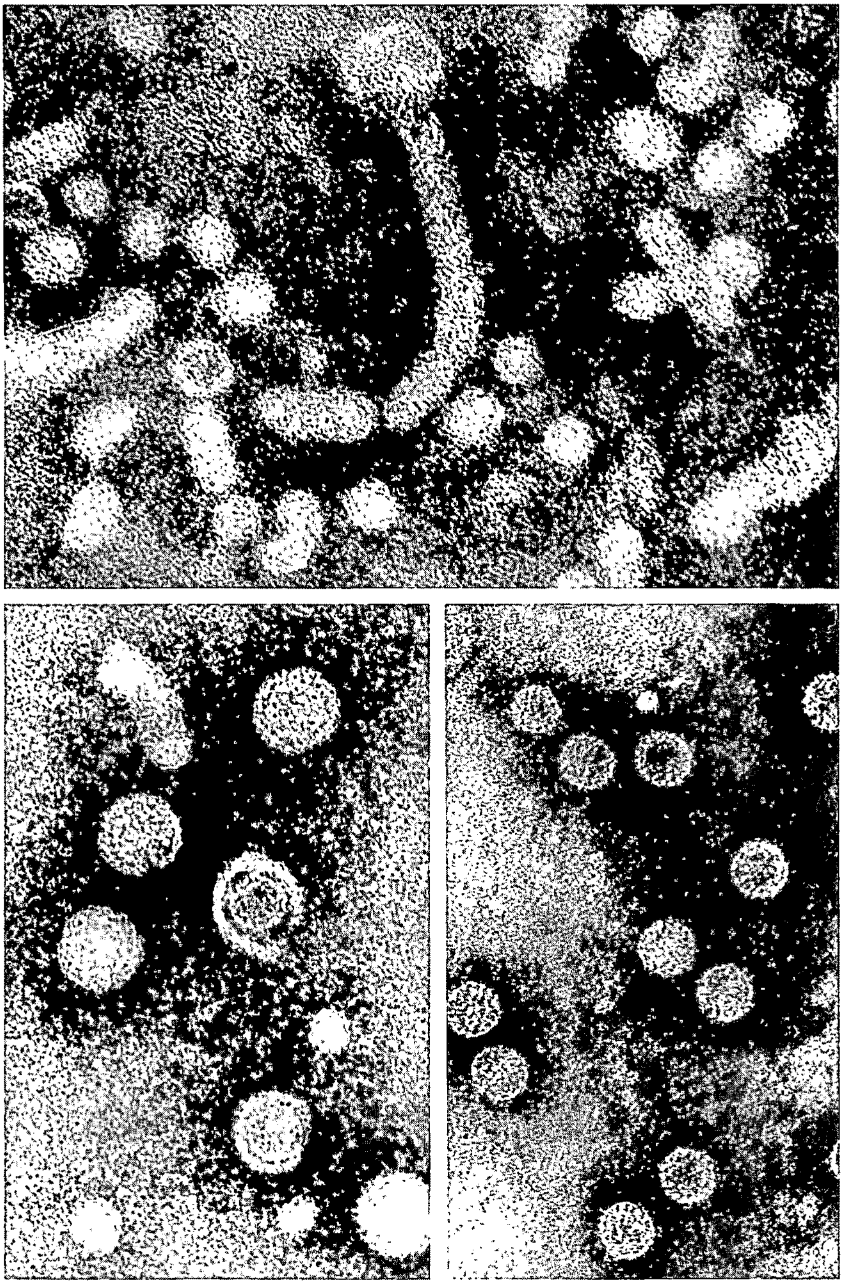
is too large for parvoviruses.] Particles of 22 nm size were found in the material of Feinstone et al, as examined by Almeida et al (17), and this may have been a parvovirus. They have also been described in human feces by Paver et al (26), but the relationship of parvoviruses to gastroenteritis in man is still nebulous. A parvovirus which has caused confusion in the context of hepatitis has been described in the serum of nine healthy blood donors and two other subjects by Cossart et al (27). It is present in the serum of healthy blood donors and anti-HBAg test sera have reacted with it because they contain antibodies to this particle also. Its significance as a pathogen is unknown, but 30% of adults in the United Kingdom possess antibodies to it. At present there is no disease consistently associated with it and the significance of possession of the virus, or of antibodies to it, is unknown. This is probably fortuitous, and there is no correlation with hepatitis A, established by Dienstag et al (28) for the 27 nm particle. It has not been grown in the laboratory, and indeed is not with certainty categorized as a virus.

## HEPATITIS B

The entity known as hepatitis B antigen (HBAg) has been thoroughly investigated and described antigenically and biochemically (29, 30). There have been several recent reviews and one comprehensive monograph (30a), and for that reason references here to the literature on hepatitis B are restricted. However, like the hepatitis A particle, it has not been grown in a cell culture system. The connection with hepatitis B is now incontrovertible. HBAg is found, usually in the blood, in acute cases of clinically and epidemiologically typical hepatitis B, if sought persistently at the right time and with a sensitive test. HBAg is also found in the sera of carriers of hepatitis B, i.e. those whose blood, on inoculation into other humans, causes an acute clinical HBAg-positive hepatitis. Transfusion hepatitis has been largely eliminated from blood volunteer donors, but much less radically from blood from paid donors.

In the first studies with HBAg it was regarded essentially as a serological entity. Electron microscopy of negatively stained preparations of sera from cases or carriers revealed a diversity of morphology, manifested in three principal forms (Figures 2 *a–c*): (*a*) Approximately spherical units of size about 18–20 nm. This is the form which appears to have been the subject of most early biochemical studies on HBAg. (*b*) Longer forms, of roughly the same diameter and surface appearance. These may be 100–1000 nm long. These are known to share a surface antigen with the 19–20 nm form. (*c*) Large, approximately spherical, double-shelled particles, resembling

*Figure 2* Hepatitis B antigen in human serum. Preparation negatively stained with phosphotungstic acid. All X 275,000. (a) Small forms (18–20 nm) and longer forms. Serum of patient with acute hepatitis B. (b) Dane particles. These show the two components (surface and core) of the particle, and one (*right, center*) has a ruptured surface layer and displays the core. (c) Cores of Dane particles (purified $HB_cAg$) from homogenate of chimpanzee liver by CsCl gradient centrifugation, treated with chimpanzee anti-$HB_c$ antiserum. Preparation similar to that illustrated by Barker et al (32a; Figure 6) Electron micrographs by June D. Almeida.



*a* and *b* in that they share with them a surface antigen, but having an inner component about 27 nm in diameter. This inner component may be exposed and made available to antibody by mild detergent treatment of the Dane particle (31). All three types of particles contain protein, lipid, and carbohydrate. Only the Dane particle contains nucleic acid. There is now a standardized nomenclature for the components of the Dane particle (Table 3; ref. 32). The Dane particle (Figure 3) has a regularity of form, at least in its inner component, and also a pattern of morphology familiar in other viruses. Its description and separation from the other two forms was made in material prepared by negative staining. It was by negative staining that liver homogenates from patients HBAg positive at death were found to contain 27 nm particles corresponding in size to the cores of the Dane particle, and apparently antigenically distinct from the other two types of particle. By the technique of thin sectioning it was shown that the nuclei of liver cells in HBAg-positive immunodeprived subjects showed spherical particles 22 nm diameter, which corresponds with 27 nm by negative staining (33). An electron microscopic study, in which immune electron microscopy was applied to the released cores of the Dane particle (31), established with certainty that the two components are antigenically distinct, and that the outer component is antigenically related to the other forms of HBAg. The Dane particle has been further characterized biochemically. It contains double-stranded DNA (molecular weight 1.6 X $10^6$) and is associated with a DNA-dependent DNA polymerase (34). The DNA itself has been extracted, and there is evidence that it replicates according to the rolling circle mechanism (35). Antibody to the surfaces of all three types of particles occurs during the acute phase of the illness, but does not persist, except in a few patients repeatedly passively stimulated (e.g. hemophilics). Antibody to the core also appears during the acute illness, probably earlier than antibody to the surface. It is found universally in chronic carriers of HBAg, and appears to constitute evidence of the replication of the Dane particle (36), either transiently as in the acute illness, or persistently, as in the carrier with chronic hepatitis. Detection of antibody to the core of the Dane particle is not easily performed, because it is necessary either to perform immune electron microscopy on detergent-treated preparations or to use the CF reaction. For this a purified core preparation is required, and this has proved difficult to obtain except from extracts of postmortem livers from chimpanzees (37).

Table 3 Nomenclature of hepatitis B antigen (32)

| | |
|---|---|
| $HB_sAg$ | antigen occurring on surface of all three forms of HBAg, including Dane particle ("surface antigen") |
| $HB_cAg$ | antigen occurring on core of Dane particle ("core antigen") |
| Dane particle | particle (~ 42 nm), containing both $HB_sAg$ and $HB_cAg$ |
| HBV | abbreviation reserved for hepatitis B virus when this is finally characterized |
| Anti-$HB_s$ | antibody to $HB_sAg$ ("anti-surface antibody") |
| Anti-$HB_c$ | antibody to $HB_cAg$ ("anti-core antibody") |

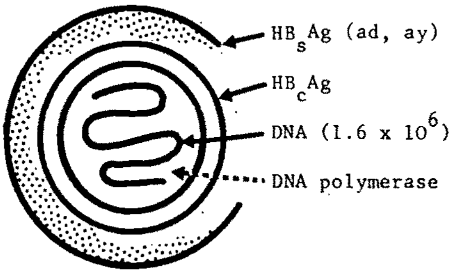


*Figure 3* Dane particle, diagrammatically represented. The outer shell contains carbohydrate, lipid, and protein. The core contains protein and DNA. The DNA polymerase activity is associated with the core, but its exact situation in the core is uncertain. Designed and drawn by Peter Lister.

The antigenic makeup of HBAg is consistent from time to time in any one patient, and from patient to patient when they are etiologically connected, i.e. in one outbreak of hepatitis B (38). There are variations from patient to patient, from outbreak to outbreak, and from one geographical location to another. All specimens of HBAg have a common antigenic determinant *a*, and in addition, one of two mutually exclusive determinants *d* or *y*. Although it has been suggested that *ay* (as opposed to *ad*) is typical of drug addicts, and is associated with frank acute or chronic liver disease, it appears much more likely that the distribution of *d* and *y* is essentially geographical. Symptomless carriers have, by and large, the serotype commonly found in their country of origin, where they acquired the HBAg, probably as a mild or symptomless infection in infancy or childhood. There are also two other subtypes, independent of *d* and *y*, called *r* and *w*, so that subjects may be *adr, ady, ayr,* or *adw; ayr* is very rare, though not unknown. These "antigens" are probably to be regarded as determinant groups rather than as separate antigens.

HBAg was detected first by the Ouchterlony double gel-diffusion test, and this remains a reliable test, although outmatched for sensitivity by some newer techniques. The significance of a positive HBAg for an individual, on the one hand, and for nursing and medical staff on the other, is so far-reaching that the production of false positives is a serious reflection on any technique. The method which at the present time combines sensitivity, rapidity, reliability, and ease of operation is the red cell hemagglutination described by Cayzer et al (39), although it is probable that in reliable hands, the radioimmunoassay can detect a few very low titer positives, e.g. artificially pooled material from a large number of donors, only one of whom may have been positive, as in some commercial preparations of antihemophilic globulin. Passive immunity can be conferred with purified and concentrated specific anti-HBAg immunoglobulin, although the prophylactic efficacy of this material has yet to be proved. Vaccines have been prepared, on a trial basis, consisting of HBAg from which Dane particles have been removed, followed by formalin treatment.

Such measures of control over hepatitis B as are possible, and these are now considerable, are based entirely on the detection of HBAg. It is significant that a large measure of control is possible in this disease, even though the etiological agent has not been isolated in the laboratory. Hepatitis B is commonly, but not exclusively, a disease seen in hospitals, and its control in hospitals has become more urgent as more complex techniques develop (40).

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Dr. June Almeida is guilty of aiding and abetting the production of this review, but is responsible for none of its shortcomings. In particular, I am indebted to her for the electron micrographs with which it is illustrated.

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# TEMPORAL LOBE EPILEPSY: NEUROLOGICAL AND BEHAVIORAL ASPECTS

❖7178

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## INTRODUCTION

It is perhaps fair to say that in no other disorder do the disciplines of neurology and psychiatry overlap so extensively as in epilepsy. Despite this, lay and at times professional organizations have mounted well-intended but unfortunate (for the patient) propaganda campaigns to deny this special association. As a consequence, the serious psychiatric complications of epilepsy have often gone unrecognized by the internist or neurologist and have generally been of little interest to the psychiatrist. A better understanding of this association is of paramount importance if we are to provide these patients with comprehensive treatment for a complex disorder.

Although the prevalence of psychical aberrations in epileptics has been recognized since ancient times (1), it was not until the advent of modern electroencephalographic (EEG) technology (2) that a special association of severe psychiatric disorders with the psychomotor (temporal lobe) form of epilepsy was documented. Interest in this association of epilepsy and in particular temporal lobe epilepsy (TLE) with various forms of behavioral disorder has produced a rich but confusing and often contradictory literature. In part this is due to the loose nosology characterizing much of the psychiatric literature and also because of the varying neurological and EEG criteria chosen for making the diagnosis of TLE. Diagnostic difficulties arise on the one hand from the protean manifestations which characterize the clinical seizures and on the other from the fact that electrical discharges deep in the temporal lobe frequently go undetected in the scalp-recorded EEG.

For our purposes, the individual temporal lobe seizures of TLE may be defined operationally as "focal seizures produced by discharging lesions situated in or [secondarily] projecting to the temporal lobes" (3). It would undoubtedly be more correct to speak of limbic rather than temporal lobe epilepsy since many clinical features appear to depend upon the propagation of the discharges from the temporal lobe per se into structures with which the temporal lobes have major anatomical connections, e.g. the hypothalamus. However, because the appellation TLE is so widely used, this terminology will be adhered to in what follows.

## TEMPORAL LOBE SEIZURES, THEIR RECOGNITION AND CLASSIFICATION

The early promise of the EEG for the study of deranged cerebral activity has been realized most fully in the area of epilepsy. However, the remoteness of temporal lobe electrical discharges from the scalp recording electrodes poses a number of difficulties for the EEG diagnosis of TLE. Similarly, neuroradiographic procedures have been of limited diagnostic value in part because of the dangers inherent in their routine use, a situation which will undoubtedly change with the wider use of computerized brain tomography. The neurological examination is also of only limited value in the diagnosis of TLE since it is usually completely normal. An infrequent but important sign is an homonymous superior quadrantanopia resulting from an injury to the temporal portion of the optic radiation. As a result of these limitations, the diagnosis of TLE at present depends primarily upon the semiology of the clinical seizures. The seizures in other forms of focal epilepsy are characterized by elementary neurological signs and symptoms (paresthesias, myoclonic movements, etc). Although temporal lobe seizures may share some features in common with other types of focal and/or generalized seizures, they tend on the whole to be characterized by complex sensory, motor, and autonomic disturbances superimposed on a background of altered consciousness. Automatisms are the most characteristic seizures seen in psychomotor (temporal lobe) epilepsy. These include licking, smacking of the lips, clicking of the tongue, and chewing and swallowing movements (4). Purposeless but coordinated movements of the hands constitute another important group of automatic movements. In general the various signs and symptoms which characterize these seizures may be grouped into four major categories: somatomotor, sensory, psychical, and autonomic phenomena. [For detailed classification see (5).] Some psychical phenomena deserve special consideration.

### *Ictal Amnesia*

The critical role played by mesial temporal structures in elaborating recent memory has been thoroughly documented (6–8). Seizures arising in these structures may be characterized by an ictal amnesia. Their shorter duration and the lack of hypermnesia for the beginning and end of the amnesic period distinguishes such fits from hysterical fugue states. One highly characteristic ictal memory disturbance in TLE is that of *deja vu* and, less commonly, *jamais vu.* The misperceptions of familiarity occur more frequently when the epileptogenic focus involves the right temporal lobe (9). Related to the *deja vu* phenomenon are those attacks originally described by Hughlings Jackson as *dreamy states.* In these, familiar objects or people may seem peculiar and unreal to the the patient. Still another ictal disorder of memory is *forced thinking.* In this type of seizure consciousness is suddenly interrupted by the feeling of a forced channeling of the patient's thoughts.

Patients with TLE may complain of poor memory (interictal amnesia) which in practice is usually difficult to document. In fact it may be that memorizing becomes defective during periods of repeated subclinical seizures.

### *Hallucinations*

Among the various psychical phenomena which occur in TLE, hallucinatory experiences are common. The most characteristic of these is the so-called olfactory hallucination or uncinate fit. The hallucinated odor typically has an unpleasant (stench-like) quality often described as disgusting and rarely if ever as pleasant (4). Formed visual hallucinations may occur and are more common when the epileptogenic focus is in the right temporal lobe.

### *Ictal Aggression*

Aggressive acts occurring as part of an ictal episode are of great interest not only because of what they might teach us about the neuroanatomical substrate of emotion and behavior but because of their medicolegal significance. MacDonald (10) indicates that while there is indeed considerable medicolegal interest in aggression associated with epilepsy, "his own experience based on a personal series of one-thousand court reports yielded only two examples of crimes committed as a result of an epileptic seizure." Knox (11) reviewed the records of 434 unselected epileptics attending a single clinic and in only one patient was peri-ictal aggressiveness a feature of the case. Gunn & Fenton (12) carefully reviewed the histories of 32 epileptic patients who were committed to the Broadmoor Hospital because of violent crimes. More than half of these patients were found to be suffering from TLE. Two patients were found whose aggressive acts appeared to occur during the immediate postictal state of clouded consciousness. Thus it seems that while aggressive behavior is an unusual ictal or peri-ictal event in an unselected group of epileptic patients, in patients with TLE, aggressive behavior may occur peri-ictally in at least 6%, and possibly more of the cases. It must be stressed that up to this point the discussion has been limited to aggressive behavior as a peri-ictal event. Interictal aggressive behavior is considered in a later section.

### *Ictal Sexual Disturbances*

Ictal sexual disturbances are rare, but disordered sexual behavior including hyper- and hyposexuality, transvestitism, and fetishism may occur in patients with TLE as a part of the seizure or interictally.

## ELECTROENCEPHALOGRAPHIC DIAGNOSIS

Much of the controversy surrounding the specificity of the relationship of TLE to various forms of behavior disorder devolves upon the diagnostic criteria used to interpret the EEG. According to Penfield & Jasper (4), "Paroxysmal high-voltage electrical potentials of almost any form or frequency may be considered epileptiform from the EEG point of view . . . ". Jasper however, acknowledged certain limitations regarding the significance of paroxysmal slow waves. He noted that they are of diagnostic significance only when they occur in close temporal relation to the clinical seizure. Although both localized anterior temporal (13), and bilateral parasaggital theta (14) slow waves may signify TLE, much confusion can be avoided by insisting on sharp or spike activity as an essential diagnostic EEG criterion.

Another source of confusion is that focal discharges arising in the deep temporal structures may not be recorded by scalp electrodes until widespread generalization of the afterdischarge has taken place. Thus a case of TLE may be mistakenly diagnosed as one of the generalized epilepsies. It cannot be overemphasized that unless these points are kept in mind, grave errors will result. This, as we shall see, is particularly true in attempting to find electroclinical correlations in patients with epilepsy and disturbed behavior.

It is estimated (15) that 20–40% of patients with epilepsy, particularly TLE, have a normal routine EEG. To assist in making the diagnosis, barbiturate-induced sleep recordings have been recommended. Our own studies (16) indicate that this has no superiority over natural sleep. Both types of sleep appear to have definite but limited value. In about two thirds of patients with chronic TLE, the EEG reveals bilateral abnormalities (17). This appears to be a function of the duration of the illness and usually does not indicate demonstrable pathology in both temporal lobes (see below). This finding of bilateral EEG abnormalities in the face of unilateral pathology has given rise to the notion of the so-called mirror focus. According to this concept, the primary epileptogenic focus (in the pathological temporal lobe) evokes a secondary contralateral (mirror) focus, by electrical bombardment across the anterior commissure. Experimental data suggest that after some period of time the secondary focus may become independent, i.e. it will persist even if the primary focus is surgically removed. In man, surgery at this stage may result in a severe memory disturbance similar to that seen in Korsakoff's psychosis (7). Both the Wada test (unilateral intracarotid injection of sodium amobarbital) and the fractional, intracarotid injection of Metrazol (18) when done with EEG monitoring may assist in determining the presence of an independent mirror focus.

The value of the EEG has been augmented by the application of computer techniques to its analysis. Such techniques have been fruitfully applied to the analysis of EEG data derived from depth electrodes, to improve electroclinical correlations (19). These studies, however, have raised new and difficult problems by showing that the spread of seizure discharges is not limited to pathways determined by patterns of normal synaptic connectivity but may in fact involve antidromic neuronal invasion.

## PATHOGENESIS

The most common neuropathological finding in patients with TLE, both at surgery and at postmortem examination, is the so-called Ammon's horn sclerosis. This is characterized by nerve cell loss and fibrosclerosis of the Sommer's sector of the hippocampus (20). In most cases this mesial temporal sclerosis (MTS) is unilateral, although the contralateral side is sometimes affected to a lesser extent. In only about 5% of the cases is the scarring of equal severity bilaterally. Although the specificity of this lesion as the cause of temporal lobe epileptic seizures has been questioned, Margerison & Corsellis (21) present compelling arguments in support of this concept, based on the clinical, EEG, and histopathological findings in 55 autopsied cases.

Nevertheless the precise mechanisms responsible for the occurrence of this epileptogenic lesion are still in question. The older view implicating intrauterine hypoxia (4) as the fundamental cause has been challenged by Norman (22), who makes a powerful case for childhood *febrile status* resulting from a congenital low seizure threshold. The correctness of one of these views vis-à-vis the other is of more than academic interest. If indeed the brain damage occurs not before but at the time of the initial convulsion, it is rational to employ extremely vigorous efforts to abort these febrile seizures of childhood as quickly as possible. The mechanism of febrile status might account for the increased prevalence of TLE in the offspring of parents with TLE. It is important to note that the other types of focal epilepsy do not appear to have a genetic component.

A very important, although less frequently reported lesion, commonly limited to surgical specimens, is a small cryptic tumor of the temporal lobe, the so-called hamartoma (23).

## INTERICTAL DISORDERS OF BEHAVIOR

Many early authors considered certain psychological peculiarities in epileptics as constituting an "epileptic personality." Although variably defined, certain personality traits form a common thread running across and binding together these early descriptions. The essential features include a day-to-day variability and unpredictability of behavior, characterized by irritability and an explosive temperament, which alternates with an unctious good naturedness. There is a heightening of egocentricity and a deepening of emotional responsiveness characterized by oceanic ideation and religious fervor. There is a pseudotalumudic approach to the analysis of trivial events characterized by verboseness both in speaking and in writing. In the clinical setting the perseverative and circumstantial nature of these productions is appreciated as a sticky or adhesive quality sometimes referred to as the glischroid or ictothymic personality. The fact that many epileptics do not show abnormal psychiatric features or personality traits has led several leading epileptologists to question the validity of this concept (24).

Subsequent correlations of the EEG findings with the clinical seizure pattern (13, 25) led to the recognition of the specific form of epilepsy now generally termed temporal lobe epilepsy. Gibbs (2) was able to use this discovery to resolve the seeming inconsistencies by showing that such interictal psychiatric disorders were far more frequent in the temporal lobe group than in all other epileptics.

### *Psychotic Reactions*

The finding of an increased prevalence of psychotic reactions among patients with TLE has been confirmed by many authors (26–30), with only occasional reports tending away from this view. One often-quoted study (31), while it acknowledges the association of psychopathology and epilepsy in general, denies any specificity with respect to TLE. Nevertheless this study reveals that "nearly two thirds of all the adult epileptics studied who were known to have had psychotic episodes also had a diagnosis of psychomotor [temporal lobe] epilepsy." This study also revealed the fact that a schizophrenic-like psychosis occurred almost twice as frequently in the TLE group compared to the so-called grand mal epilepsy group. Despite this, a number of statistical maneuvers are used by the author to deny her own data. This odd manipulation of the data notwithstanding, several fundamental conceptual difficulties undermine the principal argument in this study (17).

The case against this special association is also dealt with at length in the monograph by Guerrant et al (32). The authors have as their stated goal "to test the hypothesis that people with psychomotor [TLE] epilepsy are more disturbed psychiatrically than those with other chronic illnesses." Based on clinical psychiatric examinations they found the frequency of psychosis among their psychomotor epileptics to be five times that of their non–psychomotor epileptics. The authors tend to minimize this finding by noting that 13% of a control group made up of patients with chronic medical illnesses were said to be psychotic, certainly an unusual finding on most general medical wards. This curious treatment of their data aside, the authors reluctantly conclude, "it cannot be said that the hypothesis was completely disproved."

Thus both studies, which are often quoted as having shown statistically that there is no difference between TLE and other forms of epilepsy with respect to psychiatric abnormalities, have in fact confirmed a special association between schizophrenic-like psychotic reactions and TLE. This schizophreniform psychosis occurring in association with TLE has been detailed in a number of recent papers (30, 33, 34). An excellent analysis of the clinical features in this disorder may be found in the extensive report of Slater, Beard & Glithero (35). In their study these authors review the clinical picture in 69 patients in whom there was an association of epilepsy and a schizophreniform psychosis. Of their 69 patients, 45 were clear cases of TLE. In their patients paranoid reactions were most common while catatonic phenomena were unusual. A distinction between this disorder and true schizophrenia is the preservation of affect. The specificity of this type of psychosis occurring in epilepsy is underscored by the observations of others that the other major functional psychosis, i.e. manic-depressive reaction, rarely if ever accompanies epilepsy (36).

Some authors (37, 38) have commented on a curious inverse relationship between the severity of the psychosis and the seizure frequency. It is probably this inverse relationship between the seizures on the one hand and the psychosis on the other that lead to the mutual antagonism theory of Meduna (39). It is certainly an interesting turn of events that this intriguing "yin-yang" interrelationship between epilepsy and schizophrenia has become transformed into a theory of mutual exclusion. Landolt (40) extended this observation from the level of the clinical seizure pattern to the level of the recorded electrophysiological events (EEG) and introduced the concept of forced normalization. He used this term to describe the tendency for EEG paroxysmal activity to subside during a psychotic episode and to reappear upon termination of the psychosis. Attempts to confirm Landolt's hypothesis have produced conflicting results (31, 41). This is not surprising, since for reasons previously discussed, electroclinical correlations in TLE are notoriously apt to be misleading. This difficulty notwithstanding, the observation that psychotic episodes may be precipitated by those anticonvulsants that are particularly effective in controlling temporal lobe seizures (phenylacetylurea, primadone, and ospolot) lends force to the notion of a unique inverse relationship between TLE and psychosis. Conversely, in such patients the major tranquilizers (phenothiazines) may, while reducing the intensity of the psychotic reaction, aggravate the seizures. This observation has led to the use of chlorpromazine as a provocative test for the EEG diagnosis of TLE (42).

The mechanism subsuming this inverse relationship is not known. However, two recent findings derived from sleep deprivation studies may have relevance for this question. One recent study in man (43) suggests that deprivation of paradoxical [rapid eye movement (REM)] sleep may induce a psychotic reaction, which, although not identical to the paranoid schizophreniform psychosis of TLE, is on balance more similar than dissimilar to it. In animal experiments (44), on the other hand, induced convulsions (electroconvulsive shock) can reverse the effects of REM deprivation. Coupling these findings with the previously mentioned pharmacological effects, the following tentative formulation is suggested. Long-standing TLE may lead to a derangement in the normal functioning of the limbic system with respect to the normal expression of nocturnal REM periods. Should the limbic component of these pent-up REM periods eventually spill over into waking periods as perhaps occurs in the nocturnally REM-deprived subjects, "waking-dreaming" (hallucinations) would occur. On the other hand, the experimental data mentioned above suggest that seizures might provide an alternate outlet for this pent-up REM activity. If so, then the psychotic events would not express themselves. Thus, pent-up REM activity blocked by chlorpromazine from expressing itself as a psychotic event might be released in the form of seizure activity. Alternatively, pent-up REM activity blocked by anticonvulsants from expressing itself as seizures might be released in the form of psychotic behavior. For the present these suggestions remain, of course, in the realm of speculation and conjecture, and while they may account for this inverse relationship they do not explain how TLE might initially provoke a psychosis. However, disorders of REM sleep have been reported in patients with TLE (45).

### *Aggressive/Assaultive Behavior*

As an ictal event, aggressive/assaultive behavior appears to be a rare phenomenon (see above). Since the recognition of TLE as a distinct type of epilepsy, the older notion of episodic epileptic mania has been refined into the concept of an interictal psychopathy characterized by aggressive behavior, specifically associated with TLE.

Taylor (46) has extensively reviewed the literature on this association and commented on the frequently noted finding of a less than optimal home environment in the histories of these patients. From our own experience and numerous accounts by other authors it is clear that there are critical environmental stimuli, more or less specific in each case, which appear to trigger the angry outbursts. The nature of the aggressive behavior in these patients differs from that of the usual psychopath in that the aggressive or violent acts are not a way of life. This is not to say, however, that they appear out of the blue in a paroxysmal fashion. Usually there is some precipitant, and the temporal lobe epileptic often goes to great lengths to justify his overreaction to what is seemingly a minor provocation. Characteristically the patient will give a long-winded and convoluted explanation for his behavior. This feature, like the tendency for temporal lobe epileptics to harbor grudges, also distinguishes their aggressive/assaultive behavior from that seen in patients with frontal lobe lesions.

A prospective study in 100 children (47) with TLE has shown that if the seizures are limited to the minor spells characteristic of temporal lobe fits, aggressive behavior does not occur. If the patient also has generalized seizures, then catastrophic rage, as these authors term it, does occur. This finding suggests that the electrical discharges must propagate beyond the confines of the temporal lobe per se if aggressive behavior is to become a feature of the disorder. Based on what we know from animal studies, it is highly likely that the abnormal electrical events must involve the hypothalamic portion of the limbic system. This might account for the observation that when other types of epilepsy involve the limbic circuit (secondary temporalization), they too may lead to aggressive behavior.

The precise mechanisms and possibly multiple determinants subsuming this association are incompletely understood. Nevertheless the critical role played by an epileptogenic focus in the temporal lobe has been unequivocally demonstrated by the results of temporal lobe excision. In the large surgical series reported by Falconer and co-workers (48) more than one third of their patients with TLE had shown aggressive or violent behavior. In these patients in whom extensive drug treatment, psychotherapy, and environmental manipulation had all failed to modify either the behavior or the epilepsy, surgical ablation of the epileptogenic focus often led to dramatic improvement in the aggressive behavior when the surgery also resulted in the elimination of the seizures. This was true despite the fact that all other life factors had remained unchanged.

## CLINICOPATHOLOGICAL CORRELATION

Falconer found that in those patients in whom aggressive behavior was a significant feature, mesial temporal sclerosis was the most common lesion found at surgery. On the other hand, in those temporal lobe epileptics who developed the paranoid schizophreniform psychosis, small hamartomas of the amygdala or fusiform gyrus were the most common lesions found at surgery. Falconer suggests that this dichotomy may in some way be related to the presumed genetic basis of the hamartomas. The arguments of Taylor (46) that the type of psychopathy is related to the age of onset of the seizures rather than to the nature of underlying lesion seem more convincing. The onset of epilepsy with mesial temporal sclerosis typically occurs in infancy, while for the hamartomatous group it begins at puberty.

A major aspect of early childhood socialization is the development of inhibitory mechanisms controlling limbically mediated behavior. It is reasonable to suppose therefore that early-onset limbic epilepsy (TLE) may interfere with this process, and as a result there develops only poor control over aggressive tendencies. Such aggressive tendencies might actually be reinforced by abnormal electrical discharges (TLE) arising in the limbic system. The more sophisticated mental mechanisms on the other hand, e.g. those subsuming paranoid ideation, probably are not in operation in infancy but may be mobilized by late-onset hamartomatous TLE.

## LATERALIZATION

Maturation of the brain is reflected in the dominant EEG frequency. Normally the delta frequency of infancy gives way to theta activity in childhood and ultimately alpha activity in adulthood. Walter & Dovey (49) have shown that theta activity disappears last in the temporal regions and later on the left than on the right side. Thus the electrophysiological data suggests that initially the left temporal lobe matures more slowly than the right. Taylor (50) invokes a mechanism of differential maturation of the temporal lobes to account for the greater prevalence of left-sided foci in cases of early-onset TLE. This differential rate of maturation is also sex dependent, with the vulnerable left temporal lobe remaining at risk for a longer period of time in boys, five years, compared to only about two years in girls.

In a recently completed study of behavioral disorders in TLE (unpublished data), I found aggressive and psychotic behavior to be more frequently associated with left temporal lobe pathology, as judged by ventricular dilatation (pneumoencephalography). Larsby & Lindgren (51) in 1940 made a similar observation. They reported that in a group of "psychiatrically deteriorated" epileptics with ventricular asymmetries, more than three fourths of the cases revealed left-sided abnormalities. In my own series the majority of psychotic patients had the onset of their seizures after five years of age. It seems unlikely, therefore, that this association of left-sided TLE and severe psychopathy can be explained solely on the basis of differential maturation of the left and right temporal lobes. This is supported by the findings reported in several other studies and reviewed by Taylor (50). They demonstrate that the psychotic reactions associated with late-onset TLE also occur more frequently with left-sided epileptogenic foci, as determined by EEG.

That epilepsy originating in one of the temporal lobes should be more likely to produce disorders of thought, emotion, and behavior need not be very surprising. It has been repeatedly demonstrated that damage to either the left or right hemisphere results in a number of characteristically different, although not totally exclusive deficits. Undoubtedly the most thoroughly studied and best documented are the disorders of language which predominantly result from lesions involving the left hemisphere. Based on studies of the effects of intracarotid Amytal tests, Serafetinides (52) has shown that disturbances of consciousness are particularly likely to follow injection of the dominant (left in 95% of the population) hemisphere. Similarly, Efron (53) has demonstrated that temporal perception and the ability to judge the simultaneity of two stimuli are functions selectively disturbed by lesions affecting the left hemisphere.

If such highest functions as consciousness, perception, and speech are selectively vulnerable to lesions affecting the left hemisphere, then the suggestion that there may exist a similar vulnerability for such functions as thought, emotion, and behavior may not be totally unreasonable. Of course, even then we obviously have no explanation for such an effect, and speculations on possible mechanisms are beyond the scope of this presentation.

## CONCLUSION

The diagnosis and treatment of temporal lobe epilepsy and its associated psychopathy remains a difficult and challenging problem. Recognition of the association is an all-important first step in pursuing this problem. For the present, strict adherence to the principles outlined above remains the primary means of achieving more accurate diagnosis and more complete treatment. Expansion of the diagnostic and therapeutic armamentarium by computer-assisted X-ray and EEG techniques and all-night sleep recordings holds great promise for a fuller attainment of these goals.

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# LATE SEQUELAE OF POSTSTREPTOCOCCAL GLOMERULONEPHRITIS

◆7179

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Prior to prospective studies which were initiated with the advent of percutaneous renal biopsy, knowledge concerning the course of acute glomerulonephritis depended almost exclusively on clinical observations, often without documentation of the presence or nature of antecedent infection. Based on such imprecise criteria, acute glomerulonephritis was viewed in the 1940s as evolving to complete healing or to chronic disease in about equal numbers of patients (1, 2). However, bacteriologic, serologic, and histologic data for precise diagnosis were often lacking, and the criteria for recovery usually included little more than the absence of symptomatic disease and the presence of little or no urinary abnormalities after a few years of follow-up (3, 4). The experience with epidemic poststreptococcal glomerulonephritis (PSGN) in the 1940s, when its etiology was firmly established, suggested that recovery was the rule; the follow-up periods of observation in these studies, which were carried out primarily in military personnel, were short (5, 6).

Prospective investigations on the natural history of established acute PSGN utilizing renal biopsy material in conjunction with clinical observations were initiated by a number of groups in the middle 1950s. Reports describing the course, mostly in the adults, emanated from several centers in the early 1960s. After periods ranging up to a few years, one third to one half of the patients had not yet healed, judging by the persistence of proteinuria. However, in most patients the follow-up periods were relatively short, and in light of the minimal clinical evidence of ongoing disease, the investigators felt that subsequent healing probably could be anticipated (7–9).

Reports on the natural history in children studied by the renal biopsy technique began to appear in the 1960s. The earlier findings indicated about 50% nonhealing after only two years of follow-up, while later observations suggested that 80% or more were healed, as judged clinically (10, 11). However, glomerular abnormalities in late renal biopsies were observed in about one third of patients, frequently in the absence of urinary abnormalities. These changes, which consisted of glomerular sclerosis without continued proliferation, were interpreted generally as a form of healing (12–14).

Our own prospective studies on the natural history of PSGN, which were initiated in 1957, suggested at first that the incidence of chronicity would approximate 50%, judging by the persistence of urinary abnormalities and increased mesangial matrix in the few patients in whom biopsies were repeated a few months from onset (9). However, in the subsequent 2 to 3 years, urinalyses showed little or no protein in most patients, and renal function seemed to be unimpaired as judged by the usual clinical criteria. We then assumed, along with other observers in the field, that this was a benign disease that progressed to recovery in practically all patients.

Our observations on the course of PSGN have continued, differing from others primarily in respect to the prolonged periods of follow-up. Further, serial histologic observations have been made despite clinical evidence of apparent healing, and precise measurements of glomerular filtration rate, utilizing the clearance of inulin, have been made to assess renal function (15). Our material presently consists of 168 individuals who sustained 174 documented episodes of acute PSGN (D. S. Baldwin, M. C. Gluck, R. G. Schacht, G. Gallo, unpublished data). Seventy-six were 16 years of age or younger at the onset of their disease, and 92 were over the age of 16. The longest period of observation is 18 years from onset; 95 have been followed for more than 2 years. Two hundred renal biopsies have been performed in 118 of these patients, and have been studied by light, immunofluorescence, and electron microscopy. Glomerular lesions were evaluated for severity of proliferation according to a grading system which ranged from 0 to V. The extent of glomerular sclerosis has been graded on a scale from A to D.

At onset, hypertension was present in 89%, nephrotic syndrome in 18%, and renal insufficiency (serum creatinine exceeding 2 mg/100 ml) in 41%, evidence that these hospitalized patients were suffering from relatively severe disease. The clinical features during long-term follow-up are presented in Table 1. The data for proteinuria, hypertension, and serum creatinine are given at onset, 6 months later, and then at yearly intervals for all of the patients under observation during each successive period. Although many were observed continuously from onset, the individual patients comprising the yearly samples vary from year to year, as the maximum period of follow-up is reached in some, and as others are lost to observation but subsequently recalled, once again to be included in the study. Table 1 depicts, then, a composite representation of the clinical course of PSGN. Within 6 months, 35% of the patients no longer had proteinuria. However, proteinuria persisted in about one half of the cases in subsequent years, in trace amounts in some, but for the most part, especially in the later years, in excess of 500 mg per day. In individual instances, proteinuria was documented to reappear or increase after having subsided for several years. Persistent proteinuria was observed at a somewhat lower incidence in children, about 30% over the long term as compared to 50% in the overall group. By one year, less than 20% continued to be hypertensive. The data demonstrate, however, that hypertension reappears subsequently, and that the incidence of ele-

**Table 1** Prevalence of proteinuria and hypertension; levels of serum creatinine in the course of poststreptococcal glomerulonephritis

| Duration from onset (years) | Proteinuria | | | | Hypertension | | Serum Creatinine | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (No.)[a] | Negative (%) | Trace (%) | Positive (%) | BP > 140/90 mm Hg (No.)[a] | (%) | (No.)[a] | mg/100 ml < 1.1 (%) | mg/100 ml 1.1 to 2.0 (%) | mg/100 ml > 2.0 (%) |
| Onset | 174 | 1 | 3 | 96 | 174 | 89 | 121 | 21 | 39 | 40 |
| 1/2 | 63 | 35 | 20 | 45 | 55 | 31 | 24 | 35 | 45 | 20 |
| 1 | 68 | 50 | 12 | 38 | 62 | 18 | 20 | 70 | 30 | 0 |
| 2 | 42 | 48 | 19 | 35 | 39 | 29 | 22 | 61 | 29 | 10 |
| 3 | 37 | 38 | 22 | 41 | 38 | 45 | 14 | 57 | 43 | 0 |
| 4 | 29 | 48 | 14 | 38 | 25 | 36 | 14 | 71 | 22 | 7 |
| 5 | 33 | 48 | 21 | 31 | 31 | 48 | 15 | 48 | 35 | 17 |
| 6 | 31 | 48 | 23 | 29 | 32 | 50 | 17 | 76 | 18 | 0 |
| 7 | 25 | 48 | 12 | 40 | 26 | 54 | 14 | 79 | 21 | 0 |
| 8 | 21 | 57 | 19 | 24 | 21 | 48 | 16 | 69 | 25 | 6 |
| 9 | 17 | 41 | 18 | 41 | 18 | 50 | 11 | 72 | 28 | 0 |
| 10 | 18 | 56 | 11 | 33 | 18 | 44 | 6 | 67 | 17 | 16 |
| 11–18 | 24 | 46 | 14 | 50 | 24 | 50 | 16 | 50 | 37 | 13 |

[a] The number of different patients in whom each of the observations was made after 174 attacks in 168 individuals.

vated blood pressure reached 50% in those under observation 5 years or more. Virtually absent at 6 months from onset in children, hypertension reappeared later, and starting with the eighth year, was observed in over 30%.

Clinical assessment of renal function (Table 1) suggests that glomerular filtration was restored roughly to normal in all patients by the end of the first year, as judged by the absence of serum creatinine levels in excess of 2 mg/100 ml, excluding the 9 patients who developed terminal uremia during the first 6 months.

In Figure 1, the incidence of glomerular sclerosis grades C or D, indicating partial or complete obliteration of at least 10% of glomeruli, is plotted sequentially for comparison with the frequency of proliferation, grades II to V. Beyond 3 years from onset, significant mesangial and endothelial cell proliferation had subsided almost completely, leaving mild proliferative changes (Grade II) in only 11%. The percentage of incidence of grades C or D sclerosis increased gradually from a value of less than 25% during the first year to an incidence of 50 to 60% during subsequent years. Progressive glomerular sclerosis occurs as proliferation is subsiding, and obliteration of glomeruli progresses in the absence of any proliferation.

In 35 randomly selected patients, filtration rates were determined by the clearance of inulin at intervals ranging from 1–16 years after onset. Filtration rate proved to be within the normal range, greater than 100 ml/min, in 18 of the 35. Glomerular sclerosis (3 out of 17), proteinuria (3 out of 18), and hypertension (4 out of 18) were observed in some of the group with essentially normal renal function, but in a low incidence. By contrast, the incidence of structural and clinical abnormalities was much greater in the 17 patients (about one half of the group thus studied) whose filtration rates proved to be reduced to less than 100 ml/min, ranging down to 40 ml (Figure 2). The lack of correlation between level of filtration rate (indicated by

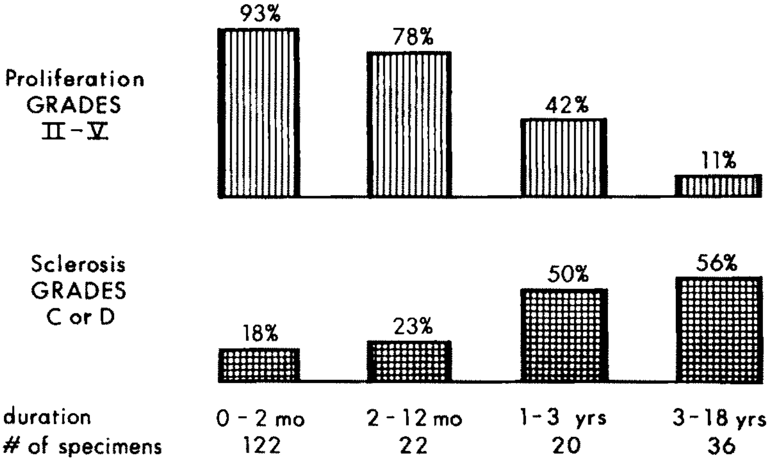
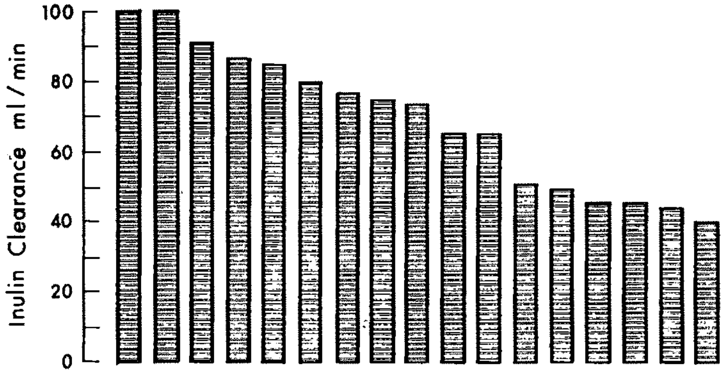


*Figure 1* Long-term course of poststreptococcal glomerulonephritis: incidence of proliferation and sclerosis.



| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sclerosis | 0 | 0 | 0 | + | 0 | 0 | 0 | + | + | + | 0 | | + | + | + | + | + |
| proteinuria | 0 | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | + | + | + | + | + |
| hypertension | 0 | + | 0 | + | + | + | + | + | + | + | 0 | + | + | + | + | + | + |
| duration | 15 | 8 | 9 | 8 | 16 | 3 | 4 | 5 | 8 | 4 | 1 | 5 | 6 | 15 | 4 | 4 | 9 |

*Figure 2* Clinical, pathologic, and functional sequelae of poststreptococcal glomerulonephritis.

the heights of the bars) and duration of disease demonstrates that the rate of progression is highly variable among patients with PSGN. In this group, glomerular sclerosis was present in 9 of 16, proteinuria in 10 of 17, and hypertension in 14 of 17. This concurrence of functional, clinical, and structural abnormalities serves to establish the interrelationship of these features of chronic disease. Viewed individually these abnormalities might appear only suggestive, but taken together, they clearly establish the presence of a glomerular disease which is responsible for functional impairment and hypertension. Interestingly, 7 of these patients with reduced filtration rates and hypertension or glomerular sclerosis had no proteinuria, demonstrating that PSGN may be responsible for obliteration of the glomerular bed and hypertension in the absence of the cardinal feature of glomerular disease, proteinuria, and in its chronic progressive stage PSGN may be clinically indistinguishable from essential hypertension.

The presence of proteinuria, hypertension, reduced filtration rate, or glomerular sclerosis has been taken as evidence of irreversible glomerular disease in assessing the occurrence of chronicity. In our 95 patients who have been followed for 2 years or more, at least one of these abnormalities is present in 60%, proteinuria in 46% (again demonstrating that irreversible disease may occur at times in the absence of proteinuria), hypertension in 42%, reduced filtration rate in 38%, and glomerular sclerosis in 50%.

What is the evidence that ultimate progression to uremia can take place? In Figure 3 the occurrence of a uremic outcome in 14 patients is illustrated. The first 9 patients in this figure followed a course characterized by severe proliferative disease and renal failure from the beginning, which never remitted, and either died

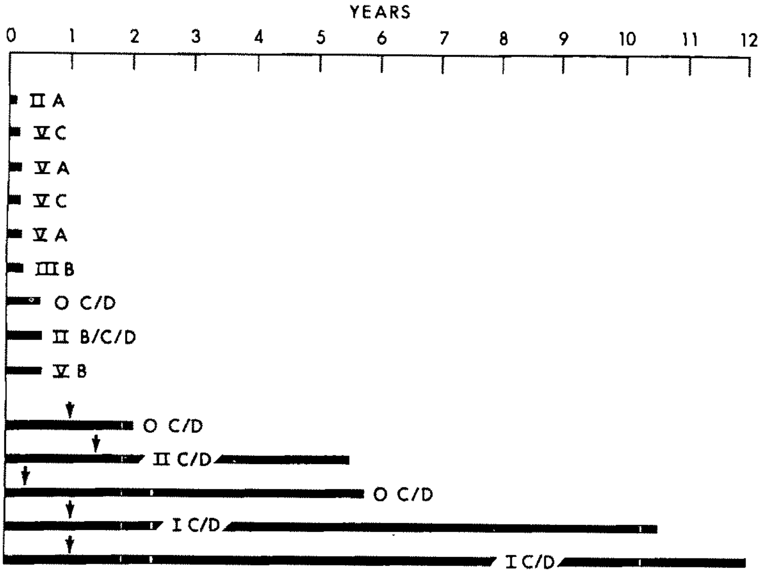


*Figure 3* Uremic outcome in poststreptococcal glomerulonephritis.

of uremia or entered hemodialysis programs within 6 months of the onset of their disease. As indicated by the symbols following the bars, the initial severe intracapillary proliferation and crescents persisted, shown as proliferation grade V, or glomerular sclerosis rapidly developed, shown by sclerosis grades C or D. That this unremitting course occasionally occurs in PSGN is well known; the incidence was about 5% in the present study. At the lower portion of Figure 3 are depicted the courses of 5 other patients who have also gone on to a uremic outcome, but along a notably different route. In these patients, at the times indicated by the arrows, glomerular proliferation had subsided and renal function had returned to normal, within the first year or so following onset. Their course was thus typical of all the other patients whose long-term course has been summarized earlier in this review. Subsequent to its early subsidence, the glomerular disease in these patients has then progressed to increasing glomerular sclerosis and terminal uremia over periods ranging from 2 to 12 years. As indicated by the symbols which follow or interrupt the bars, glomerular proliferation had subsided (shown by grades 0 to II) as sclerosis (grades C and D) was affecting more and more glomeruli.

The mechanism responsible for the progressive glomerular sclerosis which is being documented is unknown. The progress is occurring in the absence of proliferation. The C3 component of serum complement, which was reduced in three fourths of the patients at onset, returned to normal levels in all except those who died within 6 months with rapidly progressive disease. Immunoglobulin G and C3 were consistently demonstrable in glomeruli in the granular pattern typical of immune complex deposition early in the disease: 80% positive for IgG, three fourths of which were in a granular pattern, the rest linear or mixed; and 89% positive for C3. By contrast, IgG and C3 were observed less consistently in later specimens, 55% positive for IgG, 26% positive for C3, and, when present, the pattern of deposition had become linear in almost all instances.

How often the irregular glomerular sclerosis which so frequently follows acute PSGN advances to widespread glomerular obsolescence and clinically significant renal failure cannot be stated at present. Continued long-term prospective studies of PSGN and other glomerular diseases will eventually establish the relative importance of PSGN as a cause of the end-stage kidney.

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# REASSESSMENT OF HOMOSEXUALITY AND TRANSSEXUALISM

❖7180

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## HOMOSEXUALITY

The term *homosexual* has been used in the scientific, clinical, and popular literature to connote an individual's sexual behavior, sexual fantasy life, sense of inner identity, and social role. These four parameters may be congruent or incongruent in any particular individual. For instance, a man may fantasize about sex with men, engage exclusively in homoerotic acts, feel that his inner identity is homosexual, and publicly live a homosexual role. Alternatively, an individual may fantasize exclusively about males but engage in erotic activity to orgasm with both sexes. He may feel that his inner identity is homosexual but may live publicly (either single or married) as a heterosexual (1–3).

Feelings of sexual attraction toward members of the same sex are experienced by large numbers of people and often lead to sexual activity (4–6). The absolute number of such individuals in the United States totals in the millions.

An important finding of the Kinsey researchers, insufficiently emphasized, is the concept of the homosexual-heterosexual continuum. Rather than identifying two discrete populations, heterosexual and homosexual, it was noted that people fall on a continuum with regard to sexual object choice. Despite the fact that this observation is 25 years old, much subsequent speculation about etiology has implied that individuals are either homosexual, heterosexual, or bisexual (7–9).

### *Nosology*

For many years homosexual acts and feelings were regarded as pathological by mental health professionals. The Diagnostic and Statistical Manual (DSM) published by the American Psychiatric Association in 1952, lists homosexuality in the sexual deviation category, which in turn is listed under sociopathic personality disturbances. The prevailing psychiatric opinion at that time suggested an intrinsic relationship between a person's sexual object preference and conscience structure. In the more recent version of the DSM, published in 1968, homosexuality was no longer placed in the sociopathic category, but was still considered a mental disorder. In January 1974, the Board of Trustees of the American Psychiatric Association approved a recommendation of the Nomenclature and Statistics Committee that homosexuality per se be deleted as a mental disorder from the DSM (10).

This modification of nomenclature was based on the view that all mental disorders must necessarily be associated with either subjective distress or generalized impaired social effectiveness. There is no evidence that either the presence of homoerotic feelings or a history of erotic activity with the same sex must inevitably lead to distress or impair effectiveness. Nor is there substantial evidence to support the contention that there is a necessary relationship between homoerotic feelings or acts and psychopathology of any type (11, 12).

Clinical experience supports the notion that there are some people for whom homoerotic impulses are, in fact, evidence of a mental disorder. Distress may be produced because erotic impulses are incompatible with a heterosexual self-representation. Such patients would be treated with psychotherapy, psychoanalysis, or behavior therapy. Prognosis for a change of sexual object is guarded, in general. Good ego strength, a past history of heterosexual experience, and strong motivation for change are favorable prognostic signs. Individual variability is great, and each patient must be thoroughly evaluated on an individual basis. Alternatively, some persons with homoerotic feelings may be unable to cope with the difficulties of acquiring an interpersonally satisfying social niche. These patients would be counseled in a supportive manner, either on an individual basis or in a group. People in either category, according to the 1974 revision in nomenclature, are now diagnosed as having a "sexual orientation disorder."

An important point of recent emphasis is that the term homosexuality does not connote a unitary mental condition. This view was emphasized in the final report of the Task Force on Homosexuality prepared under the auspices of the National Institute of Mental Health in 1969 (13). Thus, factors motivating sexual behavior, as well as the social and emotional adjustments of individuals whose sexual preferences is for members of the same sex, are highly variable.

### *Psychodynamic Considerations*

Many psychoanalytic theoreticians have proposed that the evolution of homosexual rather than heterosexual erotic object preference stems from irrational fears of imagined danger present before the age of six which continue to unconsciously motivate behavior in the adult (14). These theories are based on therapeutic investigative work with patients (15) or other atypical groups, rather than with socially well-integrated, emotionally well-adjusted individuals of homosexual orientation. As a result, psychoanalytic theories of etiology might apply only to a deviant subgroup and not be of general relevance for the development of homoerotic feelings, thoughts, and acts.

### *Family Background*

Since prospective data are lacking, studies of family background must be interpreted cautiously with regard to the elucidation of etiology. Various types of difficulties with same-sexed parents are more commonly found in the backgrounds of males and females with significant homoerotic orientation than individuals with heteroerotic orientation (12, 15). It is not clear, however, to what degree such interpersonal problems (often assumed to lead to inadequate identifications) are causes, and to what degree they are effects. Thus a child's homosexual orientation may in some cases lead to emotional distance from an unaccepting same-sexed parent. Alternatively, a temperamental factor that predisposes a youngster to an aversion to rough-and-tumble play might also lead to emotional distance between himself and his father (16).

### *Object Relationships, Activities, and Self-Esteem*

Histories of peer group exclusion during the latency period are more common among males of predominantly homoerotic orientation than among males of predominantly heteroerotic orientation (12, 15). Such prepubertal peer group difficulties are often related to the temperamental unassertiveness and athletic awkwardness manifested by many, but not all, such youngsters. Associated with peer group difficulties, cognitive labeling may lead to a self concept in which inadequate masculinity is recognized by the self and advertised to others (17). Far less is known about the early relationships of females with significant homoerotic orientation than about males. Tomboyism is reported by Saghir & Robbins to be more common among such girls, especially tomboyism persisting into adolescence. However, the vast majority of tomboys do not mature into homosexual adults.

### *Hormonal Considerations*

Despite periodic interest, there is no conclusive evidence that hormonal factors play an important role in human sexual orientation.

There have been no well-controlled, adequately designed studies demonstrating that women with predominantly homoerotic sexual object preference are hormonally different from those whose predominant sexual preference is toward males (18, 19). Recent studies of hormonal differences between males of divergent sexual orientation have been positive in some cases (20–28) and negative in others (29–33). In all, the results of these investigations are inconclusive at present.

Genetic research continues to be of some interest. Kallman's dramatic finding of 100% concordance in sexual object preference (34) among identical twins has, however, not been confirmed by subsequent investigators (35–39).

## TRANSSEXUALISM

Transsexualism is defined phenomenologically as a disturbance of gender identity in which persons anatomically of one sex have an intense persistent desire for medical and surgical procedures that would allow them to live as members of the other sex. The urge to alter biological sex is in keeping with the individual's desire to maintain congruence between self-representation of body image and social role. Many such patients complain that they are "trapped" in the body of the wrong sex (40, 41).

### *Epidemiology*

Epidemiological data are sparse. There is some evidence that the prevalence is about one in 40,000 males (42, 43). The disorder is thought to be less common in females (by about one third to one half), but reliable data for both sexes remain to be collected.

### *Diagnostic and Therapeutic Considerations*

Incongruence between self-representation of body image and preferred social role is not considered psychotic; however, superficially the insistent belief that a "male" is really a "female" may seem to be a delusion. Transsexualism is thought to vary independently of other mental disorders and to occur in individuals of varying personality types.

Careful clinical evaluation must distinguish people who *are* transsexuals from people who merely wish to be. The wish to be transsexual may lead to behaviors that superficially appear similar to transsexualism. Such wishes, in the service of a variety of unconscious motivations, may lead to the conscious conviction of being a transsexual, which then may be conveyed with intensity to a physician. Since the medical and surgical procedures involved in sex change are radical and irreversible, the treatment team must be sure that the desire for sex change, no matter how earnestly expressed, represents an irreversible behavioral state rather than a reactive, potentially ephemeral state. Desires to alter body outline and social role may represent maladaptive solutions to intrapsychic conflict in some patients.

### *The "Sex-Change" Operation*

Clinical management of transsexualism calls for a delay period between the time of application for sex change and the performance of the surgical procedures. During this period the candidate should undergo hormonal, psychological, and social rehabilitation to the other gender role. Benjamin has advocated that this period be at least one year (44). Preliminary data from a variety of sources suggest that patients who are correctly diagnosed and managed experience significant relief of psychological distress with sex reassignment surgery (45–48). Both sexes are able to function erotically following sex change. In the female-to-male transformation, however, sexuality is not related to penile erection as it is normal males, since the artificial phallus does not have the erectile potential of the normal penis.

Laub (49) and Fisk (50) have five-year follow-up data on 93 postoperative transsexuals. By and large, those doing well before surgery are doing well postoperatively, and those with poorer social adjustments preoperatively remain poorer. Two thirds of the patients report feeling happier after surgery and about 10% report feeling worse. Additionally, Fisk has described five cases in which permitting the patient to live in the denied gender role (even without surgery) has been associated with remission from schizophrenia.

Preoperative candidates for surgery group into those whose behavior fits the classic transsexual background, those with a transvestic (fetishistic cross-dressing) history, and those with a primarily homosexual past. Follow-up data so far suggest no significant differences in the outcome of these three subgroups of "transsexual" candidates. For this reason, Fisk has grouped all classes of persons requesting sex change into a "gender dysphoria syndrome" (50).

## CONCLUSION

The correct identification of the transsexual syndrome marked a recent major advance in the behavioral sciences. Future investigative effort is needed to clarify the etiology of the syndrome and the long-term salutary effects and complications of the sex change procedure. Such research would also help clarify the mechanisms by which core gender identity becomes fixed in normal boys and girls (16, 51).

With regard to homosexuality, the changes in the DSM during the last 25 years have reflected altered psychiatric perspective about the relationship between sexual orientation and psychopathology. The factors that determine sexual preference are not entirely clear at present. Their identification awaits future investigation.

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# MEDICAL AND SURGICAL TREATMENT OF THE WOLFF-PARKINSON-WHITE SYNDROME

❖7181

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## INTRODUCTION

Few chapters of electrocardiography have made such rapid progress in the last decade as the Wolff-Parkinson-White (WPW) syndrome. The introduction of new techniques (1, 2), or of older ones, such as electric stimulation of the heart, made safe for clinical-physiological investigations (3, 4), is responsible for this breakthrough and illustrates Carl Ludwig's dictum: "Die Methode ist alles." A relatively simple hypothesis appears to be adequate for an understanding of many morphological ECG aspects of this syndrome, the genesis of arrhythmias, and the influence of drugs on their causative mechanisms. What follows is a bird's-eye view (5–8).

## ANATOMIC MATRIX OF WPW; PROPERTIES OF ANOMALOUS PATHWAYS

The anatomical matrix which describes the possible mechanisms (9) is still conjectural in many respects. While other mechanisms are possible, it may be stated that the classic WPW syndrome, for all practical purposes, is caused by a mechanism based on the presence of one, or rarely two, conducting anomalous pathways of small dimensions (diameter 1 mm or less, length ½ to 1 cm?). They connect, anatomically and electrophysiologically, adjacent parts of atrial and ventricular muscle situated at opposite but contiguous parts of the atrioventricular (av) ring and at any part of it (8), with the exception of the region of direct contact of aortic and mitral valves. They can often conduct in both directions (a–v, v–a), sometimes only in one of these (mostly in the va direction). Variations of an anatomical nature (location, probably diameter and length of the anomalous pathway) and of electro-

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physiological characteristics of the normal and abnormal structures involved may complicate this relatively simple situation.

### *Mechanisms; the Tachycardia Initiating Interval*

Circus movement tachycardia may only be initiated in that part of the cardiac cycle in which only one av connection can conduct (tachycardia initiating interval). This interval is the electrophysiological Achilles heel of the WPW heart. The longer the duration of the tachycardia initiating interval, the greater is the chance that a premature beat falling in this interval causes a circus movement tachycardia (atrial premature beat–av node/His–ventricles–anomalous pathway–atria (2), or ventricular premature beat–anomalous pathway–atria–av node/His–ventricles). The first form of tachycardia (85%) does have normal QRS form and duration. In the second, rare type, the complexes show pre-excitation. In some patients, permanent antegrade block in the anomalous pathway is present with intact retrograde conduction: here a ventricular premature beat may cause a circus movement tachycardia. The circus movement tachycardia can often be terminated by one atrial premature beat or ventricular premature beat, elicited in or near the pathway of the circus movement (4), the basis for the treatment with pacemakers.

## MEDICAL TREATMENT

The careful examination, indicated in every patient with tachycardia, should also consider emotional factors, which are very important in the genesis of tachycardia of any type. The delineation of av node function by the single-test stimulation technique, presently in use, and often with heavy sedation of the patient and the injection of local anesthetics, suggests a too-deterministic response pattern. Emotional factors (anxiety) greatly influence its function. It is therefore not surprising that the elimination of psychological problems may cure tachycardias resistant to drug therapy.

### *Pharmacological Prevention and Treatment*

Without adequate diagnosis of the tachycardia it is difficult to develop a rational treatment program. This can be done with long-term ECG tape recording (10). The effect of treatment is sometimes followed by electrophysiological investigations but this type of investigation is only indicated if the treatment is not effective. The relation between electrophysiological effect and serum level of drugs injected intravenously may be different from that existing after long-term oral application (11). Moreover, digoxin, propanolol, quinidine, and procainamide hydrochloride are important examples of drugs exhibiting great variability in dosage requirements (11). Optimal results with drugs can only be obtained if the patient has "ritualized" its use, adequate dosages are given, and preferably long-acting preparations are used. For the prevention of attacks one could try to make the initiating mechanism less effective by suppression of premature beats and reduction in duration or, even better, abolishment of the tachycardia initiating interval. Quinidine and procainamide hydrochloride (12, 13) are probably the best drugs in this respect, but no results of continuous observations are known as yet and their effectiveness is not clearly established, but seems likely on clinical grounds. In some patients the net effect of their action on the refractory period and probably on conduction velocity is a shift of the tachycardia initiating interval to the right, without changing or even lengthening its net duration. In this situation a premature beat occurring later in the cardiac cycle than before treatment may still cause a tachycardia. The dangers of long-term use of procainamide hydrochloride are well known and regular control is necessary.

If, as is often the case, the refractory period of the anomalous pathway is longer than that of the av node/His, this difference may be decreased by digitalis, which shortens the former (10–50 msec) and prolongs the latter (30–70 msec) (14). Because of its relative safety, propanolol (15) is well suited for preventive treatment; because of its lengthening effect on the refractory period of the av node/His, it does not significantly influence the refractory period of the anomalous pathway and so can be given in combination with digitalis.

## TREATMENT OF ATTACKS OF TACHYCARDIA

Treatment of tachycardia may be very frustrating. The cycle length of the tachycardia can be increased by all drugs which reduce conduction velocity somewhere in the cardiac structures directly involved in the circus movement. The excitation wave will be blocked if the duration of the refractory period in one of the constituting structures of the circus pathway exceeds the duration of one cycle of the circulating wave. However, all known drugs with this pharmacological action also increase the duration of this cycle by decreasing conduction velocity. Great interindividual variability in response to pharmacological interventions is often present, as may be expected from the differences in size of the circus and in the degree of participation of atrial and ventricular muscle and of the specific conduction system. Up to 50 mg of ajmaline, a rauwolfia alkaloid not available in the United States, injected intravenously in 5 minutes, decreases or may even block antegrade conduction through the anomalous pathway in many instances (13). Procainamide hydrochloride has the same effect if given in a dosage of 100 mg intravenously every 5 minutes, until either the ventricular rate slows down sufficiently (16), one gram is given, or untoward side effects appear. These drugs are particularly indicated in atrial fibrillation or flutter with rapid ventricular rate. Here digitalis should not be given if it has not been used in the similar situation in the same patient, because in several instances ventricular fibrillation has followed its injection in these conditions.

### *Surgical Treatment*

A very small group of patients cannot be helped adequately by medical treatment, and their prolonged, repetitive episodes of tachycardia seriously interfere with normal life (17). Even more desperate are the patients with long-lasting attacks of atrial fibrillation or atrial flutter with extremely rapid heart rate, up to 300/min—due to 1:1 conduction—which cannot be controlled sufficiently. They often have complicating heart disease of various kinds as an additional burden. Sometimes syncope does occur in these situations, which according to Sealy (18) must be considered a surgical emergency. In these rare situations surgery is an ultimum refugiens, because it is a major risky procedure, with numerous prerequisites difficult to fulfill.

## INVESTIGATIVE PROTOCOL

### *Preoperative*

The main objectives of preoperative investigations are as follows:

1. The demonstration that an anomalous pathway is directly involved in initiation and maintenance of the tachycardia present in this patient; the bypass must be a part of the tachycardia circuit or be responsible for the rapid heart rate in atrial fibrillation or flutter. Of great importance is the study of the initiation of the tachycardia responsible for the symptoms (4, 8). The influence of atrial and ventricular premature beats of different origins on initiation and termination of the tachycardia must be studied systematically. The refractory period of the anomalous pathway for conduction in both directions should be determined, because a short refractory period for antegrade conduction is a necessary but not a sufficient condition for a rapid ventricular rate. This investigation can only be done with sophisticated and safe electrical stimulators, His bundle, and multiple intracavitary and body surface ECG recordings. In a few instances the arrhythmias do originate in the same way as in the normal hearts, without direct participation of the anomalous pathway, e.g. an intranodal circus movement may cause a tachycardia and the role of the anomalous pathway in this situation is secondary (7, 19).
2. The possible location of the anomalous pathway in the heart, of importance for the surgical approach. The Rosenbaum criteria (20) may be misleading when the contribution of the delta wave to the WPW complex is small. The contribution of the delta wave to the fusion complex can be increased during atrial premature beats. One might see the gradual transition of a type B complex in $V_1$ to a typical type A complex. Pre-excitation will also be increased during stimulation of that part of the right or left atrium as close as possible to the origin of the anomalous pathway. During circus movement tachycardia with narrow QRS complexes, the region of earliest atrial activation (due to retrograde anomalous pathway conduction) indicates the atrial origin of the anomalous pathway. The conclusion of localization is clearly based on indirect evidence; the electric activity of the anomalous pathway cannot be recorded as yet. The differentiation between septal and lateral localizations is very difficult, if not impossible, before the operation.
3. The detection of concomitant heart disease, its severity, and possible indications for correction.

### *During the Operation*

Surgery should never be done without intimate knowledge of the methods for exploration of epicardial and intramural excitation (8, 21). Many difficulties and pitfalls may occur, such as the disappearance of pre-excitation or the occurrence of atrial fibrillation with complexes showing different degrees of fusion and corresponding differences in epicardial excitation times of the anomalous pathway activated region. Tachycardias must be elicited or stopped during the operation. The localization of earliest epicardial activation, preferably performed during predominant or exclusive pre-excitation, is not necessarily the region of influx of the anomalous pathway. A septal localization must be considered, if the earliest epicardial breakthrough region is situated near the anterior or posterior attachment of the ventricular septum and occurs after the beginning of the delta wave in body surface leads (8). Surgery has been ineffective several times, as demonstrated by the not infrequent persistence of the WPW syndrome after surgery for attempted ablation. One reason may be the presence of two anomalous pathways. Several successful interventions have been reported (8, 18, 22–25).

## SURGICAL METHODS

The method described by Sealy et al (18, 26) is the best one available. During cardiac bypass the atrial wall near the atrial end of the anomalous pathway is incised from inside out, until the fatty tissue surrounding the coronary vessels in the av groove is reached, then the two parts are sutured again. This operation, even if performed by the experts at Duke University, is not always a complete success, defined as disappearance of the delta wave and of the attacks of tachycardia. This did happen in 18 of the 30 collected surgical cases with one postoperative death (26). In 6 the delta wave was modified but persisted, although tachycardias were abolished or markedly diminished. In 3 there was no effect on either the delta wave or the tachycardias; in 2 the delta wave disappeared, but recurrent tachycardias remained; another patient, who never demonstrated a delta wave, died soon after operation.

In the near future it will be necessary to study the relation between the form and polarity of the delta wave in body surface leads and the location of the anomalous pathway, the details of anomalous pathway conduction during sinus beats and atrial and ventricular premature beats, and the relation between size of bundle and the occurrence of ventricular fibrillation during atrial fibrillation and flutter. The WPW syndrome has changed from a "budget of paradoxes" which could not be solved until recently to a field of increasing investigative activity which has already resulted in the possibility of ultimate cure for its life-threatening potentialities.

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# CORONARY ARTERY SPASM: A NEW APPRAISAL

❖7182

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## INTRODUCTION

When this author was a medical student in the early 1940s, the prevailing concept of the mechanism of angina pectoris was that it was due to intermittent spasm of coronary arteries. It was not regarded as a serious, potentially deadly illness since it was so readily relieved by nitroglycerin. This opinion was no doubt based on the firm assertion by Sir William Osler (1) during his second Lumlian Lecture on Angina Pectoris to the Royal College of Physicians in March 1910, when he said, "Spasm or narrowing of a coronary artery . . . may so modify the action of a section of the heart that it works with disturbed tension, and there are stretching and strain sufficient to arouse painful sensations. I do not know of any better explanation of anginal pain."

Then in 1940 the paper by Blumgart, Schlesinger & Davis appeared (2) in which they showed by postmortem coronary injections of X-ray contrast material that patients who had clinical angina during life had in most instances extensive narrowing and often complete atheromatous occlusion of one or more coronary arteries. These findings indicating that angina pectoris is a serious manifestation of a potentially life-threatening disease dominated the concept of angina during the next thirty years and as late as 1968 this author wrote in an article that the work of Blumgart and his associates had "laid to rest the concept that angina was due to coronary spasm . . ." (3).

## RECOGNITION OF VASOSPASTIC ANGINA

In 1959 Prinzmetal (4) published his paper on variant angina in which he described patients who had typical anginal pains at rest but not with exercise. Their ST segments on ECG became elevated during pain rather than depressed as in effort angina. Both types were relieved by nitroglycerin. Prinzmetal postulated that the episodes were due to "increase in tonus" of a coronary artery or *spasm.*

Variant angina was looked upon as an interesting rarity of not much clinical significance until one day in 1970, when something happened before the eyes of this author that appeared to throw new light on the genesis of anginal pain. He wrote the following in the chart of patient M. H., a 56-year-old woman who had just had a bypass saphenous vein graft for treatment of Prinzmetal's variant angina:

> During the surgical operation when the right coronary artery was being exposed it went into a state of intense spasm so that the entire length of the exposed artery beyond the area of the angiographically identified local stenosis became hard and rope-like. At this time the ECG (lead I) monitor disclosed ST segment depression as the heart action weakened and the blood pressure fell to 70/60. Nitroglycerin was given under the tongue and in a minute the blood pressure rose and the ECG improved, the ST segments coming back to the isoelectric line. At the same time it was noted that the spasm of the artery was relaxing and a soft pliable section of the vessel could be identified suitable for vein grafting. . . . There were no further problems with coronary spasm during the remainder of the operation.
>
> These observations made on the exposed heart raise the intriguing question whether vasospasm has been playing a role in the pathogenesis of the variant angina syndrome of which she is a classic prototype. Could it be that the non-occlusive stenotic lesion in the right coronary artery somehow acts as a trigger area for setting off episodes of vasospasm? The disturbing clinical implication of these observations in this woman is that the bypass operation might not help her if arterial spasm really is a factor and if that spasm can extend beyond the point of anastomosis of the graft.

One week later she reported recurrence of typical nocturnal angina episodes of mild intensity. These were subsequently documented by ECG monitor. They have continued at infrequent intervals during the last 5 years, but they have never been intense as before and they have been quickly relieved by nitroglycerin. A repeat catheterization showed a closed vein graft and a persistent 60% stenosis of the right coronary artery.

This landmark case appears to prove that coronary spasm is indeed a mechanism capable of producing severe myocardial ischemia, for the electrocardiographic and hemodynamic consequences of myocardial ischemia were being demonstrated by our patient during spasm even though she could not experience the angina under anesthesia. The observation appears to substantiate Prinzmetal's hypothesis that coronary spasm is the cause of variant angina.

Over a period of 10 years MacAlpin, Alvaro & Kattus (5) were able to collect a series of 20 patients with typical variant angina. We were able to show that the majority of these had excellent exercise tolerance on treadmill testing, most of them without ischemic ECG changes, yet angina attacks arising out of sleep or repose were accompanied by marked ST segment elevations when 12 lead ECGs could be obtained during pain. In some with single lead monitoring only ST depression was found, but this could have been reciprocal to elevation on the other side. In 18 out of 20 we found local stenotic lesions on coronary angiography, but in two there were no obstructive lesions at all. The frequent association of the local stenotic lesion with the vessel supplying the intermittently ischemic myocardium suggests that the lesion has something to do with triggering off the episodes of spasm. Yet well-documented episodes of rest angina have been reported frequently in patients with coronary arteries free of obstructive disease as we have seen in two of our series (5, 6–10).

Coronary artery spasm has been recognized for years by angiographers as a transient local phenomenon due to a tactile stimulus by the tip of the catheter to the inner arterial wall (11). This has usually been localized to the proximal portion of the vessel near the point of contact and has been regarded as clinically unimportant since it was almost entirely asymptomatic. However, since our direct-vision observation of coronary spasm at the operating table, a number of dramatic accounts of spontaneously occurring coronary spasm documented by coronary arteriography have appeared. Dhurandhar (12) was the first to describe coronary spasm accompanying a typical episode of anginal pain with relief of pain associated with the opening of the constricted segment. Since the spasm was close to the catheter tip, there is some suspicion that the spasm might have been artificially induced. The observation of Oliva, Potts & Pluss (13), however, is completely convincing since the spasm involved and closed the entire right coronary artery, which then reopened and closed again without any chance of stimulation by the catheter tip. Pain and relief of pain coincided with closing and opening of the spastic vessel. The cases described by King et al (14), Hart et al (15), and Kerin & MacLeod (16) are confirmatory and leave no doubt about the validity of the presence of coronary spasm as the mechanism of Prinzmetal's variant angina.

## SEARCH FOR THE INCITING CAUSE

What has not been determined is the inciting cause or trigger mechanism that sets off the spasm. Catheter tip spasm, well known to angiographers, is clearly a tactile stimulus, and in the instance in our operating room the surgeon was palpating the wall of the coronary when it went into spasm, also a tactile stimulus. But how can this play a role in the spontaneously arising spasm? Could the local stenosis present in most cases give rise to the equivalent of a tactile stimulus? In the one occurrence that we have photographed in our laboratories the spasm extended proximally from the permanent 60% narrowing in the mid-right coronary artery. In the operating room case it extended distally. In the literature, spasm has been reported to encompass a stenotic area or to extend to either side of it. Thus, local stenosis does seem to play a role, but how it does so is unknown.

The theory that attacks might arise out of disturbed sleep or nightmares as suggested by Scherf & Cohen (17) is not tenable, since we have questioned a number of patients as they wakened from sleep with nocturnal angina and they have reported deep dreamless sleep. This issue is eloquently settled by the remarkable achievement of Masari (18) of Pisa, Italy, who was able to record left ventricular hemodynamics during 26 episodes of spontaneous angina in five patients. The duration of ST segment elevation was 20 to 380 seconds. One half of the episodes were accompanied by ventricular ectopic beats either singly or in salvos. Pain always began after the onset of ST elevation, but some episodes of ST elevation occurred without pain. He states, "In no instance was the onset of ST elevation preceded by increase of heart rate, systolic pressure or ventricular peak dp/dt which could indicate a rise of myocardial metabolic demand. The first noticeable event after the onset of ST elevation was an obvious fall of the relaxation or contraction peak dp/dt. Left ventricular systolic pressure fell and end diastolic pressure increased from 4 to 16 mmHg." Coronary spasm with complete vessel closure was documented angiographically in four of the five patients. In no case was mechanical stimulation involved because the catheter tip was not introduced until after the onset of ST elevation. Three cases had local stenotic disease and two did not. Thus, Maseri's report would indicate that vasospastic angina is due to an abrupt cutoff of the blood supply to a large segment of myocardium with ECG and physiologic consequences resembling the changes seen on ligation of a canine coronary artery (19). Increased metabolic demand is not a factor. No increase of systemic neurohumoral activity can be identified as the triggering mechanism.

## POSSIBLE TRIGGER MECHANISMS

My colleague, MacAlpin, and I have attempted to induce attacks in three patients with variant angina by the infusion of sympathomimetic and cholinergic drugs both before and after alpha and beta blockade. All attempts were unsuccessful, as was the application of the cold pressor test. Gensini (20) has produced angiographically demonstrable occlusive coronary spasm in dogs by infusion of pitressin. Wilson & Johnston (21) reported an early case of variant angina in which attacks could be repeatedly reproduced by having the patient inhale cigarette smoke. I have received a verbal report from a colleague who described a woman in whom coronary vasospasm could be reproduced by arousing her anger. Leachman (22) and his associates have reported long-term relief of variant angina by reserpine therapy, suggesting that intracardiac catecholamine release might play a role.

Perhaps the most illuminating observation comes from Lange et al (23) who studied an outbreak of Sunday night–Monday morning rest angina among workers in a nitroglycerin-cellulose rocket propellant plant who developed nocturnal angina upon withdrawal over the weekend from the industrial environment of nitroglycerin dust with its strong vasodilating properties. Obliterative coronary spasm was documented angiographically over long segments of vessel in patients experiencing angina during the withdrawal. It was postulated that the strong coronary vasodilation stimulus experienced during the 5 days of exposure to nitroglycerin was followed during the weekend withdrawal by reactive hypertonus sometimes strong enough to produce complete vasospastic coronary occlusion.

There may be another clinical expression of this mechanism. For example, a fireman recently performed a near maximal treadmill exercise test increasing his heart rate to 170. He remained symptom free while doing the test and no ischemic changes appeared in the ECG. Twenty minutes later he experienced severe chest pain while taking a tepid shower. An anterolateral myocardial infarction evolved, from which he recovered. Reactive hypertonus of a coronary artery following a maximal vasodilator stimulus is suggested by the time sequences of this episode. We saw similar time sequences in two other cases in which the onset of pain followed an unaccustomed maximal physical effort by 20 to 30 minutes.

If this reactive hypertonus mechanism does play a role, then why does it happen so rarely? The inference might be that there is a particular individual sensitivity of the coronary arteries to vasomotor stimuli that varies in intensity from time to time. Hope that the phenomenon will be possible to study in more detail has been raised recently by publication of a report by Clark et al (24) that injection of ergonovine maleate will reliably induce coronary vasospasm in susceptible patients, frequently reproducing attacks of angina. We have recently succeeded in documenting such an induced coronary spasm in our angiographic laboratory. Such spasms have all been reversible by administration of sublingual nitroglycerin.

## OTHER CLINICAL IMPLICATIONS

One cannot escape the suspicion that coronary spasm may be the mechanism that underlies some of the mysterious aspects of more common clinical entities. Myocardial infarction following permanent closure of a coronary artery is readily understandable, but infarction of areas supplied by arteries that are wide open or only partially occluded must be due to temporary coronary occlusion, probably vasospasm. Khan & Haywood (25) have recently reported a series of cases and reviewed this subject. Furthermore, the suspicion has recently been raised that coronary thrombosis is not the principal cause of myocardial infarction. Roberts & Buja (26) have pointed out that patients who die early from heart attacks rarely have thrombi in the coronary arteries. They suggest that the thrombus is the result rather than the cause of the attack. What, then, is the cause? The classic concept is that coronary blood supply is insufficient to meet the metabolic demand of the myocardium. Since most myocardial infarcts begin during rest or repose it is difficult to blame increased demand. Reduced supply by vasospastic closure is a possible explanation. If so, then early and vigorous application of vasodilator therapy, previously believed to be potentially harmful by lowering blood pressure, might indeed be highly effective in aborting or minimizing the severity of the infarct.

In angina pectoris of effort it may be vascular hypertonus that explains some of the puzzling features. Why is it that some patients have pain on first effort of the day but none on similar activity later on? Why will a golfer have angina on the first or second hole but none thereafter? What is it that permits the angina patient to perform so much better on a second test effort than on the first, and why is it that some can walk right through an angina episode (27) without stopping?

## CONCLUSION

Coronary spasm appears to be proven as the mechanism of Prinzmetal's variant angina. It is probably the cause of myocardial infarction without coronary occlusion. It may play a subtle role in conventional myocardial infarction and also in exertional angina. The inciting mechanism for triggering vasospastic angina attacks is not known, but clinical observations suggest that there may be a multiplicity of factors as well as individual susceptibility. Now that coronary spasm can be induced by ergonovine maleate in man it may be possible to study mechanisms more readily and thus find methods of control.

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# HUMAN DISEASE ASSOCIATED WITH EXPOSURE TO LIGHT

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## INTRODUCTION

In contrast to the beneficent effects of most of the solar spectrum, short-wave ultraviolet (uv) light may harm man and his environment in a variety of ways. The positive effects of wavelengths between 290 and 320 nm—the *erythemogenic* or UVB portion—are confined to a few easily substituted actions, such as photooxidation of intracutaneous 7-dehydrocholesterol to Vitamin $D_3$, and as therapy for a few skin diseases. Other medical and biological roles of light, ranging from treatment of neonatal hyperbilirubinemia to the photosynthesis of plants, require visible or at most modest amounts of long-wave ultraviolet—the wavelengths between 320 and 400 nm, also called black light or UVA, which cannot alone injure skin. The dangers of exposure to UVB deserve reemphasis at a time when human technology in the form of high-flying jet aircraft (1) and aerosol spray propellants (2) threatens to dissipate the major shield against solar ultraviolet flux provided by stratospheric ozone. These hazards include direct injury to proteins within enzymes and membranes (sunburn), and to nucleotides (skin cancer, plant mutations), as well as indirect damage when ultraviolet rays combine with exogenous chemicals or intrinsic disease states. Comprehensive reviews of most of these areas have recently been published in book form (3).

### *Direct Injury: Sunburn*

Only rays with wavelengths less than about 320 nm possess sufficient quantal energy—3.9 to 4.2 eV (4)—to cause erythema on normal human skin, while the ozone filter excludes from the earth's surface almost 100% of rays shorter than 290 nm. This critical band of wavelengths, termed UVB, is thus responsible for almost all of the injurious consequences of light exposure, although longer wavelengths may enhance such injury in vivo (5) and can apparently kill fibroblasts in culture through generation of toxic photoproducts from riboflavin (6). Thresholds and extent of damage depend on more than the quality and amount of light received, however.

Transparency of the skin's uppermost layer, the stratum corneum, changes with hydration (increased by high humidity, or immersion), with pigment content (racial and genetic influence), and with regional differences in thickness.

Unless an abnormal photosensitizer intervenes to involve tissue lipids, the major primary photochemical injury in acute sunburn reactions involves protein and nucleic acid. Indirect evidence suggests that both structural (membrane) and enzymatic proteins are early targets. A general reduction in both protein and RNA synthesis can be detected early in the period following injury (7). While lysosomes appear microscopically intact (8), their contents are released to the cytoplasm within minutes of exposure (9). Aromatic amino acids, especially tyrosine and tryptophan, are propelled into electronically excited states on interacting with an ultraviolet photon, then release this energy either by direct, radiationless transfer to a neighboring molecule, or by forming a free radical-hydrated electron pair able to act at a slightly greater distance (10). The result may destroy function in two ways; either by affecting tertiary structure by breaking nearby cystine disulfide bonds, or by creating new and aberrant covalent crosslinks leading to insoluble brown polymers (11).

Direct photochemical damage occurs to DNA and RNA in a comparable manner, with pyrimidines some ten times more sensitive to uv photons than purines. Here the resultant monomolecular (hydration, oxidation, reduction) or bimolecular (dimerization, aberrant crosslinks) damage can often be repaired by processes discussed below, though mutation or cell death may follow severe injury.

The links between primary chemical changes and the signs which include the redness, heat, and pain of clinical sunburn remain unclear. After the first few hours, much of the syndrome can be accounted for by massive synthesis and release of E-series prostaglandins, whose precursors, the $\omega$-6 essential fatty acids, appear to become available from membranes through the action of lysosomal and other phospholipases (12). Although the cutaneous pain (13) of sunburn may in part depend upon activation of kinins (14), or lipoperoxides (15), the effectiveness of corticosteroids, and especially of aspirin and indomethacin, both inhibitors of microsomal prostaglandin synthetase, in counteracting the erythema and discomfort, tends to suggest that PGE plays the major role. But PGE may act primarily on cutaneous vasodilator nerves rather than directly upon vessels, since clinical reports indicate that denervation of the skin of the extremities renders the anesthetic area impervious to sunburn (16). Although topically-applied indomethacin dramatically reduces the redness and hyperalgesia of sunburned skin, at least when applied immediately following injury (17), it fails to influence damage to protein or DNA and thus may provide a false sense of security against late effects.

### *Direct Injury: Cutaneous Neoplasia*

Both epidemiologic studies in humans and experimental production of tumors in animals confirm that exposure to erythemally-effective UVB is a dominant cause of cutaneous neoplasia (18). The evidence is strongest for squamous cell carcinoma, adequate for basal cell epithelioma, and highly suggestive for melanoma (19). In animals a single heavy dose of UVB, administered without other manipulations, proved capable of causing malignancy (20), while increasingly precise population surveys have added reliability to *Dubreuilh's* 1907 observation that city workers of Marseilles were less likely to develop the disorder than were workers in the open vineyards of nearby Bordeaux (21). The recent Third National Cancer Survey established that by age 50, 1% per year of all white males in the area surrounding Dallas, Texas developed skin malignancy (22). By age 80 the figure was 3% per year. Both rates were double those of more northerly Minneapolis-St. Paul and outranked all other cancers combined. As with acute sunburn, these tumors localize to sun-exposed sites and occur more frequently in non- or poorly-pigmented individuals.

### *Indirect Injury: Lack of Protective Pigment*

Deficient pigmentation leads to excessive sensitivity to sunburn injury, to grossly visible dermal connective tissue changes at an early age, and to a higher than normal rate of development of cutaneous neoplasms. A modified classification of genetic forms and clinical features of albinism is presented elsewhere (23, 24). Patients with vitiligo, phenylketonuria, or localized loss of pigmentation due to injury or inflammatory skin disease also run enhanced risks of cutaneous cancer. In the Vogt-Koyanagi-Harada syndrome, an acute, presumably viral disease, the skin and hair around the eyes turns white soon after an episode resembling aseptic meningitis. Ocular inflammation leads to photophobia and loss of uveal pigment, sometimes accompanied by dyscoria and deafness. Widespread cutaneous pigment loss reminiscent of vitiligo follows the ocular symptoms. No cause is known, although Fitzpatrick has referred to a cluster of cases in Chihuahua, Mexico suggesting an infectious origin (25).

### *Nutritional Disorders—Pellagra*

The dermatitis of pellagra may follow mild heat or friction, but often follows exposure to ultraviolet light in tropical and subtropical areas. The affected areas at first become red and indurated and may later develop bullae and erosions. In the classic adult form the erythema progresses to hyperpigmentation, hyperkeratosis, and a characteristic heavy desquamation, at first revealing normal-appearing pink skin beneath. The face is often affected less severely than the extremities and anterior chest, but the reason for this selectivity is unclear. The borders of the affected area are often prominent on the chest, giving rise to a necklace-like appearance (Casal's necklace). The perineal area becomes raw, eroded, and scaly. Stomatitis, gastroenteritis, and neurologic manifestations complete the clinical picture.

Although overdependence on a diet of corn or similar sources of protein with low biologic value and imbalance of amino acids is less common in developed countries today than formerly, occasional cases due solely to undernutrition still occur. In parts of the world where kwashiorkor is prevalent it is a common error to attribute to that disease the cutaneous signs of pellagra. In India pellagra develops in those who must live exclusively on millet (*Sorghum vulgare* or Jowar), a grain with adequate tryptophan but a very high leucine content. One mechanism of the leucine effect may be interference with synthesis of the coenzyme forms of niacin, the di- and triphosphopyridine nucleotides (26). In developed countries the pellagrin is often a chronic alcoholic, has a primary illness marked by severe debility or malabsorption, or has a defect in tryptophan utilization or absorption such as a massive carcinoid tumor (27), or Hartnup's syndrome. Pellagra-like changes are also occasionally seen following prolonged ingestion of semipurified diets developed for the treatment of inborn errors of metabolism (28), though the tissue changes are probably due to more complex, combined deficiencies. Finally, a few patients given isoniazid (INH) without pyridoxine supplements have developed pellagra (29). Pyridoxine is a required cofactor in synthesis of niacin from tryptophan, but INH may also compete with niacin for incorporation into the active coenzyme diphosphopyridine nucleotide. Mercaptopurine and presumably azathioprine can also produce pellagrous sun sensitivity, though rarely so (30).

## *Connective Tissue Diseases*

LUPUS ERYTHEMATOSUS (LE) The adverse effects of sunlight on both systemic and cutaneous lupus are well established. In large series approximately one fourth to one half of patients with systemic lupus erythematosus (SLE) will have adverse reactions to normally tolerated levels of uv, while twice that fraction of patients with discoid LE will have symptoms (31). Exacerbation of existing skin lesions, fever, and progression of systemic activity may occur after excessive exposure; however, sunlight is not an essential part of the pathogenesis of LE. Many SLE patients tan well, occasionally even sustaining accidental sunburn without relapse, though the best medical advice would include instructions for taking reasonable care against overexposure.

Recent use of quantitative phototesting techniques has answered the question of which wavelengths cause skin injury in LE. These studies indicate that an action spectrum up to 330 nm—slightly above the sunburn range for normal persons—will produce persistent erythema, indurated plaques, and other LE-like lesions in approximately one third of patients with SLE (32, 33). The older suspicion that such responses represent a nonspecific result of trauma may be correct in some instances; lesions of discoid LE may develop at sites of nonspecific injury. In other patients, however, very large amounts of long wave uv and visible light (equivalent to heat) could be applied without causing similar lesions (32). One group has found that the characteristic marker for LE-specific inflammatory activity, the band of immunoglobulin deposited at the basement membrane between epidermis and dermis, appeared quite late in the course of evolution of a lesion induced by uv light (33).

The ultimate pathophysiologic basis of photosensitivity in LE is incompletely understood. The absence of subepidermal immunoglobulin until late in the course of uv-induced skin lesions (33), together with the absence of antibodies specific for uv-altered DNA in the sera of photosensitive patients with SLE (34) makes it seem more likely that the uv-induced skin lesions in this disease result from damage caused by thymus-derived lymphocytes, activated perhaps by DNA released from uv-damaged skin. Most attempts, however, to detect cellular hypersensitivity in LE patients by intradermal injections of DNA, or by in vitro studies of lymphocytes challenged with DNA (reviewed in 35) have failed to relate the presence of DNA-sensitive lymphocytes to the course of the disease.

## *Reactions Due to Photosensitizers*

ENDOGENOUS COMPOUNDS Porphyrins are the only photosensitizers definitely synthesized internally. Since disorders marked by excessive synthesis of these compunds, some of which cause severe photosensitivity, have been reviewed recently (36), they will not be considered in detail. Their adverse effects at the body surface are due to phototoxic enhancement of damage done by light of the long ultraviolet region of the spectrum near 400 nm, well beyond the sunburn zone. This action spectrum correlates closely with the peak of porphyrin absorption at 405 nm. The mechanism for this phototoxic effect is likely to involve direct photoexcitation of the porphyrin molecule to one of its excited states followed by free radical formation and attack on nearby sensitive sites as discussed earlier for aromatic amino acids.

The most historically interesting of these disorders is the rare recessively-inherited erythropoietic porphyria. Illis (37) has convincingly proposed this disorder as the scientific underpinning for venerable superstitions regarding the existence of werewolves and other mythical fusions of animals and man. These stories were familiar in classical Greece, have been recorded in the literature of five continents, and reached their height during the later Middle Ages. Illis was able to find examples of a number of werewolves occuring in the same family. Using medieval and subsequent sources he built up a composite picture of the average werewolf: he was usually an excessively hairy creature with pale greenish or yellowish skin, a red mouth and unsteady eyes. The skin was often described as being covered with sores or excoriations. His behavior was often disordered. The patient with erythropoietic porphyria fits this description rather well. As an autosomal recessive disorder, it was likely to occur in siblings born to consanguinous parents. Sufferers often had red teeth and red urine, suggesting that they feasted on blood. Pallid in hue due to hemolytic anemia, they suffered severe photosensitivity with scarring, hyperpigmentation, even mutilation, often followed by hypertrichosis, the whole effect suggesting an animal more than a man. Under the circumstances their tendency to venture forth only at night in a fear-ridden fashion seems understandable, both as a wish to avoid further solar damage and as a reluctance to face the suspicions of their neighbors.

## *Exogenous Causes of Photoinjury*

PHOTOXIC DRUG REACTIONS (38) The pioneer in this field was Raab, whose studies on paramecia in 1900 opened the door to photobiologic principles of critical importance. He noted that acridine orange could be added to the organism's bath in the absence of light without harm. Exposing the organism to uv without the dye was almost innocuous. However, the dye plus light application caused a lethal reaction, one which did not occur if the dye was irradiated before adding it to the bath. Oxygen was shortly found essential for the reaction, implying that peroxide formation was involved in the mechanism of damage. The term phototoxic was applied to human medicine in the late 1930s when Epstein observed reactions analogous to those of paramecia after irradiating the skin of patients given intradermal injections of sulfanilamide.

**Table 1** Major groups of phototoxic agents in man

| | |
|---|---|
| Sulfonamides | Coal tar |
| Sulfonylureas | Anthracene |
| Benzothiadiazides | Pyridine |
| Phenothiazines | Acridine |
| Tetracyclines | Phenanthrene |
| | Plant products |
| | Hypericin |
| | Psoralens |
| | Dyes |
| | Methylene blue |
| | Eosin |
| | Rose bengal |

Table 1 presents the major classes of drugs with photoxic properties. Many of these are resonating compounds, most of which are capable of fluorescence.

PHOTOTOXIC REACTIONS WITH NUCLEIC ACID The mechanisms by which light and drugs interact with nucleic acids include several listed in Table 2. Elegant studies have been published which examined interactions of plant-derived psoralens with nucleic acid. Unlike other phototoxic drugs, psoralens do not depend upon oxygen for their damaging effects. A non-damaging, loosely associated complex forms when psoralen is added to a solution of DNA in the dark, but when the solution is irradiated with the active longer uv wavelengths, the psoralen reacts covalently to form a 4-carbon, cyclic addition product with pyrimidines, but not purines of DNA (39). Certain members of the psoralen class also interact with RNA-bound pyrimidines, but the importance of this effect is unclear. If a photon scores a second hit on the complex, these psoralen monoadducts react rapidly and specifically to form diadducts crosslinking the two strands of DNA (40). This second reaction appears to proceed at least as fast as the first, and presents a defect which is only slowly repaired by a unique mechanism. This repair pathway, also

**Table 2** Ultraviolet-induced alterations in nucleic acids

| Type of alteration | Example |
|---|---|
| Involving individual pyrimidine bases | Photohydration |
| | Reduction |
| | Oxidation |
| Involving pyrimidine-pyrimidine interaction | Intrachain |
| | Interchain |
| Involving adducts between pyrimidine and non-nucleic acids | With protein (amino acids) |
| | With amines |
| | With alcohols |
| Involving chain breaks | |

important for cross-links caused by nitrogen mustard and mitomycin C, evidently requires partial excision of one strand on both sides of the crosslink, then an exchange-pairing between the remaining strand and the appropriate region of a strand of the homologous duplex chromosome, then excision and repair synthesis of both one-strand regions by conventional means (41).

### *Mechanisms of Injury from Photoxic Agents*

Porphyrins as well as phototoxic drugs differ from innocuous compounds both in possessing greater susceptibility to excitation by uv photons, and in their ability to maintain a relatively long-lived electronically excited condition termed the triplet state, whose importance lies in its persistence for $10^{-3}$–1 sec, allowing chemical as well as electronic interactions to occur with neighboring molecules.

Study of photohemolysis of both normal and porphyric red blood cells has provided valuable information for understanding the mechanisms of phototoxic injury. Normal nonporphyric cells display one peak of hemolysis at 280–300 nm and a second one at approximately 400 nm. The first zone is the characteristic area for absorption by tyrosine and tryptophan and the second is presumably due to small amounts of riboflavin and porphyrin (42). The cells of porphyric patients contain markedly increased amounts of protoporphyrin and a correspondingly enhanced sensitivity at 400 nm. A number of constituents of the red cell membrane sustain damage in these experiments: (*a*) unsaturated fatty acids undergo progressive peroxidation (42), (*b*) aromatic amino acids and cystine disulfide bonds are damaged (43), and (*c*) most important, perhaps, cholesterol undergoes one or more photochemical changes. This important structural constituent of membranes efficiently forms cholesterol-5 $\alpha$-hydroperoxide when attacked by porphyrin-generated singlet oxygen (44). Once generated, the hydroperoxide is capable of hemolyzing normal red cells added in the dark, in part perhaps by oxidizing proteins and lipids within the membrane. Besides oxidizing neighboring molecules, this compound can react internally to form the $5\alpha$, $6\alpha$ epoxide (also called cholesterol-$\alpha$-oxide), a proven cause of sarcomas when injected subcutaneously into experimental animals (45), and a compound recently isolated from uv-irradiated (but non-porphyric) mammalian skin (46). The proposal that this epoxide of cholesterol may account for ultraviolet-induced cutaneous neoplasia seems unlikely, however, in view of the absence of an increase in tumor formation in patients with erythropoietic protoporphyria.

### *Exogenous Compounds: Photoallergic Drug Reactions*

Advances during the past decade have rendered these puzzling clinical problems understandable in terms of cell-mediated immune reactions. Other modes of immunologic injury have not yet been detected in this setting. This area has been reviewed thoroughly (47) and will not be discussed in detail.

The role of light appears to relate solely to a photochemical reaction which structurally alters the haptenic group or alters its avidity for the carrier protein, which then forms the complete photoantigen. This mechanism differs from that proposed for cell-mediated immune reactions in ordinary contact dermatitis only by the intervention of long-wave uv light.

Table 3 indicates that, like phototoxic compounds, photoallergenic ones are resonating structures of low molecular weight, most of which fluoresce. The list is a long one, with halogenated antiseptic compounds used in soaps, cosmetics, and other consumer products justifiably at the top of the list. Careful quantitative studies (48) have shown that under conditions of normal use of these products, sensitive individuals, even those of dark-skinned racial background, become vulnerable to painful erythema after less than five minutes spent in Texas noonday sun (control Caucasians minimal erythema time: 20 minutes).

### *Persistent Light Reactivity*

Most patients who experience photoallergic contact dermatitis clear promptly with avoidance of antigen and light, but a small minority experience continued outbreaks whenever exposed to light even though they shun contact with the photoallergen. These patients have been called persistent light reactors (49). They may become seriously disabled, since they cannot tolerate even brief exposures to sun, daylight from the sky, or even some sources of artificial light. Their pathologic sensitivity extends over a much broader range than that of any other disorder; they are abnormally sensitive to the sunburn spectrum, and also react to long-wave uv and visible light. Their range of sensitivity is thus much broader than the action spec-

**Table 3** Major groups of photoallergenic agents in man

- Antibacterial compounds
  - Halogenated salicylanilides and related compounds
- Antibacterials
  - Sulfonamides
  - Nalidixic acid
- Antifungals
  - a) Griseofulvin
  - b) Fentichlor (UK)
  - c) Multifungin (UK)
  - d) Jadit (UK)
- Antihistamines
  - Promethazine (Phenergan)
- Blankophores (fluorescent brighteners)
- Cyclamates
- Diuretics
  - Thiazides
- Oral hypoglycemic agents
  - a) Tolbutamide (Orinase)
  - b) Chlorpropamide (Diabinese)
- Sunscreens
  - Substituted benzoic acids
- Tranquilizers
  - a) Chlorpromazine (Thorazine)
  - b) Chlordiazepoxide (Librium)
  - c) Quinidine

trum of the photosensitizer which originally triggered their reaction. This feature has led to the speculation that some new antigen-reactive system must be involved. Recent work has shown, however, that one of the most potent photocontact sensitizers, tetrachlorosalicylanilide, may continue to produce new free radical-hydrated electron pairs while remaining covalently bound to tissue protein over an extended period (49). The possibility that a persistent sensitizer accounts for the syndrome remains a viable one.

Occasionally changes resembling cutaneous lymphoma develop in severely affected patients with photocontact dermatitis if they experience continued exposure to the antigen and light (50). It is not clear to what extent this syndrome may occur in the absence of photocontact sensitivity, although most series point toward a single entity (51).

## *Diseases Marked by Defects in Repair of DNA*

XERODERMA PIGMENTOSUM Laboratory and clinical information concerning this disorder has been extensively reviewed (52), as have recent advances in understanding DNA repair mechanisms in normal cells (53). Those few patients with xeroderma pigmentosum (xp) who display normal excision repair have recently been shown (54) to perform poorly in post-replication repair (53). At least one other human disease appears to involve defective repair of light-exposed DNA: Fanconi's anemia. The limited studies available (55) implicate reduced activity in an exonuclease which is needed for removal of the damaged strand of DNA once the endonuclease—which is reduced in most patients with xp—creates a nick.

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# MEDICAL MANAGEMENT OF MYOCARDIAL INFARCTION

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## INTRODUCTION

Several new therapeutic approaches have been designed to protect jeopardized ischemic myocardium (1). Despite their promise, their effect on overall mortality may be limited by high pre-hospital mortality and low long-term survival even in patients resuscitated from ventricular fibrillation (2); and the importance of the extent of vascular disease as a determinant of long-term prognosis (3).

### *The Dynamic Nature of Myocardial Infarction*

After experimental coronary occlusion, infarction appears to evolve slowly. After 45 minutes, many cells within the ultimate infarct remain viable (4). Reperfusion within several hours protects some regions otherwise destined to undergo necrosis (5) and several other interventions favorably modify the ultimate extent of infarction when instituted early (6). Analogous results have been demonstrated in patients (7). Accordingly, several interventions have been designed to favorably influence the evolution of infarction by improving the balance between myocardial energy requirements and supply. These include reduction of ventricular afterload, and augmentation of myocardial oxygen supply or substrate availability (8–10). Other approaches, such as those designed to reduce lysosomal hydrolase activity in ischemic tissue (11), have not yet been explored as thoroughly.

### *Detection of Infarction*

The diagnosis of infarction has been based traditionally on history, serum enzyme elevations, and evolution of localized electrocardiographic changes. Recently, serum creatine phosphokinase (CPK) isoenzyme determinations have proven useful since the MB CPK isoenzyme in man is found almost exclusively in myocardium (12–14). Other biochemical indexes of infarction are being evaluated, such as elevated serum myoglobin, detected by radioimmunoassay (15), and anti-myocardial antibodies (16). However, myoglobin from the heart and from skeletal muscle cannot yet be differentiated, and antibodies lack specificity as criteria of infarction.

Infarction has been detected by scintigraphy with $^{99m}$Tc (Sn) pyrophosphate (17) and $^{99m}$Tc-tetracycline (18). The combination of scintigraphy and analysis of MB CPK improves detection and reduces the incidence of false positive diagnosis of infarction (19), particularly among patients admitted late after the onset of infarction or in those undergoing coronary artery bypass surgery.

Positron emission transaxial tomography is a potentially useful new diagnostic tool (20). Cross-sectional computer reconstructed images of the heart can be obtained after intravenous injection of short-lived, positron-emitting, cyclotron-produced physiological substrates of myocardium (e.g. $^{11}$C-palmitate). In contrast to conventional scintigraphic techniques, superimposition of myocardium on a two-dimensional image is avoided.

## *Evaluation of Left Ventricular Function*

HEMODYNAMICS Because central venous pressure and left ventricular filling pressure may be discordant (21), assessment of cardiac output and pulmonary artery occlusive pressure (PAo) with a Swan-Ganz balloon-tipped thermodilution catheter is often essential. Although normal PAo is $< 12$ mm Hg, higher pressures (14–24 mm Hg) are frequently required to sustain cardiac output in patients with infarction (22), or to differentiate hypovolemic from cardiogenic shock (23). Changes in PAo reflect altered hemodynamics more rapidly than radiographic indexes of left ventricular failure. The rare complications of bedside cardiac catheterization, including hemoptysis, dysrhythmia, and pulmonary thromboembolism, can be generally avoided with appropriate precautions.

NON-INVASIVE TECHNIQUES Left ventricular ejection fraction and stroke volume have been evaluated echocardiographically. However, localized dyskinesis impairs reliability and suitable echocardiograms are obtainable in less than 60% of patients (24).

Ejection fraction, mitral regurgitation, and intracardiac shunts can be estimated serially with radionuclide techniques utilizing ECG gating and intravenous injection of $^{99m}$Tc labelled albumin, pertechnetate, and red blood cells (25, 26). Reproducible estimates of ejection fraction have also been obtained with intravenous injections of $^{113m}$In detected at the bedside with a precordial probe (27).

## *Estimation of the Extent of Ischemic Injury*

Infarct size has been estimated from ST-segment elevations on multiple lead precordial electrocardiographic recordings (ST mapping) (28). After experimental coronary occlusion, epicardial ST-segment elevation is related to the distribution of myocardial injury, correlates with changes in surface leads, and reflects altered myocardial perfusion or oxygen requirements (6). Changes in precordial ST maps in patients reflect spontaneous extension of infarction (29), or protection of myocardium induced pharmacologically (10, 30). Although ST mapping is rapid, noninvasive, and inexpensive, it is not applicable to all patients; it may be influenced by drugs, electrolyte shifts, or pericarditis; and it reflects ischemia rather than infarction per se.

Quantification of infarct size with current scintigraphic techniques is impaired by: (*a*) superimposition; (*b*) resolution; (*c*) border recognition; and (*d*) dependence of tracer accumulation on heterogeneous blood flow in ischemic zones. Thus, despite the diagnostic sensitivity of scintigraphy, its use in quantifying infarct size is limited.

Infarct size has been estimated enzymatically from serial CPK changes in hourly serum samples (31) since myocardial CPK depletion after experimental coronary occlusion correlates with infarct size (32), corresponds to early ST-segment elevation (6), and correlates with serial serum CPK changes analyzed according to a simple mathematical model. In patients, enzymatic and morphologic estimates of infarct size correlate closely (33). The ultimate extent of evolving infarction has been predicted with the use of curve fitting techniques applied to early serial CPK changes (34), and effects of interventions on myocardial viability have been evaluated by comparing projected CPK values to those observed after implementation of the intervention (7, 35). Unfortunately, the long interval required to obtain best-fit curves from early CPK changes prior to the intervention may mask its efficacy, since the evolution of infarction may already be virtually complete.

## THERAPEUTIC APPROACHES

Management of hospitalized patients with myocardial infarction was initially directed toward maintenance of blood pressure and urine output. Subsequently, it incorporated prophylaxis and vigorous treatment of malignant ventricular dysrhythmia with consequent marked reduction of mortality. Most recently, therapy has been directed also toward protection, and, if possible, salvage of jeopardized ischemic myocardium, based on two concepts: that infarct size is an important determinant of prognosis, and that the ultimate extent of infarction can be modified favorably by early interventions.

It is now clear that infarct size is one important determinant of prognosis. Thus, (*a*) patients who succumb from infarction complicated by cardiogenic shock exhibit extensive myocardial necrosis (36); (*b*) the high six month mortality rate associated with large infarcts contrasts with a low (<5%) six month mortality rate seen with small infarcts (37, 38); (*c*) patients surviving without cardiac decompensation exhibit much less apparent myocardial damage than those surviving with impaired ventricular function (38); and (*d*) a poor prognosis is presaged by impaired ventricular function, which in turn is correlated with infarct size (39–41).

It is also clear that infarct size can be limited in experimental animals, and preliminary analogous results have been obtained in patients (7, 10). Although it is not yet clear whether protection of myocardium will alter long-term patient survival, therapeutic nihilism is not justified. The situation may be similar to that in patients with essential hypertension; high mortality was recognized and antihypertensive drugs were available long before improved survival with treatment was demonstrable.

In patients with myocardial infarction the balance between myocardial oxygen supply and demand is favored by several general measures, including (*a*) regulation of fluid and electrolyte balance; (*b*) control of cardiac rate, rhythm, and atrial transport function; (*c*) reduction of arrhythmogenic stimuli such as excess catecholamine or free fatty acid levels; (*d*) avoidance of hypoxemia, anemia, alkalosis, or acidosis; and (*e*) management of concomitant disorders such as hypertension, diabetes mellitus, or thyrotoxicosis.

In addition to these general measures, several specific therapeutic approaches have been developed to protect jeopardized myocardium, maintain ventricular performance, and support hemodynamics (Table I) (41a).

### *Agents with Positive Inotropic Effects*

DIGITALIS GLYCOSIDES After experimental myocardial infarction complicated by left ventricular failure, digitalis glycosides diminish ischemic injury, presumably since myocardial oxygen consumption ($MVO_2$) is reduced by diminished left ventricular volume and wall tension. However, digitalis increases $MVO_2$ and augments ischemic injury after experimental infarction without failure because of its effect on contractility.

After experimental coronary occlusion, acetylstrophanthidin fails to improve ventricular performance initially, but improves it after one week (42). The absence of early effects may be due to insensitivity of ischemic tissue to digitalis, the already maximal stimulation of contractility by circulating catecholamines, or dissipation of ventricular contraction in transiently dyskinetic areas (43).

Toxic electrophysiological effects of digitalis are common early after the onset of myocardial infarction, especially when hypokalemia is present. Peripheral vasoconstriction exerting an increased cardiac burden may be induced by rapid administration of digitalis glycosides intravenously (44). In addition, digitalis does not appear to alter the outcome of cardiogenic shock (23). Thus, administration of digitalis to patients with myocardial infarction entails definite risk with little likelihood of substantial benefit unless the drug is being used to treat specific dysrhythmias.

$\beta$-ADRENERGIC AGONISTS Although $\beta$-adrenergic agonists such as isoproterenol hydrochloride improve ventricular performance, they increase $MVO_2$ and lactate production (45) and augment the extent of experimentally induced infarction (6, 46).

Norepinephrine may increase $MVO_2$ even more markedly because of its vasoconstrictor as well as positive inotropic effects. As with isoproterenol hydrochloride, malignant dysrhythmias may be precipitated, leading to further deterioration of cardiac function and viability.

Congeners such as dopamine and Dobutamine with cardioselective ($\beta$-1) and predominantly positive inoptropic effects may be useful in patients with decreased cardiac output and increased PAo. Fortunately, Dopamine exerts alpha adrenergic vasoconstrictor effects only at rather high doses and the vasoconstriction is blockable with $\alpha$-antagonists. However, its prominent non-adrenergic renal arterial vasodilatation and its cardiotonic action generally improve hemodynamics and renal function (47, 48). Dobutamine, an investigational drug, is similar and increases cardiac output without substantial changes in mean arterial pressure or heart rate in experimental animals (49). Perhaps because of its relatively modest chronotropic

Table 1 Potentially useful therapeutic interventions[a]

| Hemodynamic category[b] | Mean PA[c] | PAO[c] | Suggested interventions | Remarks |
|---|---|---|---|---|
| 1. Normal | $\leq 15$ mm Hg | $\leq 12$ mm Hg | None | — |
| 2. Hyperdynamic state | $\leq 15$ | $\leq 12$ | $\beta$-adrenergic blockade | Heart rate is usually increased |
| 3. LV failure: | | | | |
| Mild | $\geq 15$ after volume repletion when hypovolemia is present initially | $\leq 18$ | Diuretics | Dyspnea, hypoxemia, or mild pulmonary vascular congestion |
| Severe | $\geq 15$ | $> 18$ | Diuretics + vasodilators | With severe pulmonary vascular congestion and pulmonary edema, positive pressure ventilation may be useful |
| 4. Cardiogenic hypotension or shock | $\geq 15$ | $\geq 18$ after volume repletion when hypovolemia is present initially | Vasodilator therapy. Circulatory assist may be useful | Cardioselective agents with positive inotropic effects such as Dopamine may be particularly useful |
| 5. Hypovolemic hypotension or shock | $\leq 15$ | $\leq 12$ | Repletion of vascular volume | Hemodynamic reclassification may be necessary after PAO is adequate |
| 6. Acute cor pulmonale | $> 15$ (PA systolic $\leq 50$) | $\leq 12$ (normal in the face of increased PA pressure) | Correction of hypoxemia and conventional management of underlying cause | Usually due to isolated or concomitant pulmonary embolism |

[a] Modified from (41a).

[b] Patients in all categories require adequate analgesia, usually best accomplished with i.v. morphine.

[c] Pulmonary artery and pulmonary artery occlusive pressures.

effects, Dobutamine improves left ventricular performance in patients without augmenting enzymatically estimated infarct size (50).

GLUCAGON After experimental infarction without cardiogenic shock, glucagon increases the contractility of non-ischemic myocardium, improves left ventricular performance, and decreases ventricular irritability (51).

In patients, glucagon (70 μg/kg) may improve ventricular performance, increase A-V conduction, and accelerate A-V nodal automaticity (52), but it does not appear to improve survival in patients with cardiogenic shock. Side effects, especially nausea and vomiting, occur frequently. Thus, except for specific indications such as pharmacological antagonism of excessive β-adrenergic blockade, glucagon is not generally useful for treatment of acute myocardial infarction.

Unfortunately, agents with positive inotropic effects have not altered the outcome of severe left ventricular failure or cardiogenic shock, and may precipitate malignant dysrhythmias and augment infarct size. However, preliminary findings indicate that recently available cardioselective β-adrenergic agents may reduce preload and improve ventricular function and coronary perfusion at a relatively low oxygen cost.

### *Therapy Designed to Decrease Myocardial Oxygen Requirements*

β-ADRENERGIC BLOCKADE β-Adrenergic blocking agents have been employed to preserve ischemic myocardium by decreasing $MVO_2$. Among patients with uncomplicated infarction, propranolol decreases cardiac index, stroke volume, and mean arterial pressure while increasing PAo only slightly. Myocardial lactate extraction increases and chest pain is often relieved (53). Benefit is most apparent in patients with sinus tachycardia, high circulating catecholamine levels, and elevated cardiac output without elevated PAo. β-Blockade has been administered only with considerable trepidation to patients with bradycardia or impaired left ventricular function (54). However, when β-blockade effectively suppresses tachyarrhythmia, improved ventricular function often results. When the prevailing heart rate is high, ventricular dysrhythmia responds poorly to lidocaine and other conventional antiarrhythmic agents. Thus, initial administration of β-blocking agents may be advantageous.

β-Blockade in patients decreases ST-segment elevation in surface electrocardiograms (30). In addition, it decreases mortality during the first 24 hours after infarction and for as long as two years, judging from results of large, randomized prospective studies (J. M. Barber, Belfast, personal communication). Tachycardia per se increases infarct size in conscious dogs (46). In concert, these findings suggest that β-blockade may preserve myocardium. Coupled with recent observations indicating that β-blockade is relatively safe even when left ventricular function is impaired, particularly when accompanied by hemodynamic monitoring, these findings justify its use in patients with rapid heart rate, persistent pain, or progressive infarction (Table 1).

REDUCTION OF VENTRICULAR AFTERLOAD PHARMACOLOGICALLY $MVO_2$ depends on left ventricular wall stress which in turn is proportional to peak-developed ventricular pressure and volume. Accordingly, patients with myocardial infarction complicated by hypertension, congestive heart failure, or mitral regurgitation have been treated with vasodilator agents to decrease impedence to ventricular ejection and reduce $MVO_2$ (Table 1). The need for hemodynamic monitoring with serial determinations of cardiac output and PAo in patients treated with vasodilators cannot be overemphasized since improvement of myocardial energetics based on this approach requires:

1. Reduction of impedance to ventricular ejection
2. Maintenance of effective coronary perfusion pressure
3. Reduction of ventricular volume sufficient to lower wall stress without lowering output
4. Avoidance of reflex tachycardia

With judicious use, administration of vasodilator agents improves hemodynamics, mollifies refractory left ventricular failure, and appears to protect ischemic myocardium.

TRIMETHAPHAN, NITROPRUSSIDE, AND NITROGLYCERIN Reduction of ventricular afterload with intravenous infusions of *trimethaphan,* 100–1000 μg/min in patients with acute myocardial infarction associated with acute or chronic hypertension protects myocardium (7) and reduces one month mortality. Trimethaphan is less likely to produce reflex tachycardia than direct acting vasodilators because of its mechanism of action—ganglionic blockade.

Intravenous infusion of 10–100 μg/min of *nitroprusside* increases cardiac output, decreases PAo (55, 56), decreases $MVO_2$, relieves chest pain, and diminishes the frequency of premature ventricular complexes (PVCs) (56). Improved cardiac output occurs even in patients with cardiogenic shock, in whom arterial diastolic and coronary perfusion pressure is maintained by external counterpulsation (57).

Similar results have been obtained with phentolamine hydrochloride, an $\alpha$-adrenergic blocking agent (55, 58). Unfortunately, its expense is prohibitive.

Despite traditional reluctance to administer *nitroglycerin* in the face of acute myocardial infarction, it has been employed recently, based on some provocative experimental findings, including augmentation of endocardial and collateral coronary flow (59), increased cardiac output and decreased $MVO_2$ (60), and increased threshold and decreased incidence of spontaneous ventricular fibrillation (61). Salutary effects on dysrhythmia, myocardial CPK depletion, ST-segment elevation, and infarction detected morphologically were potentiated by maintenance of diastolic pressure with methoxamine hydrochloride (62).

In patients with low PAo, venodilatation induced by nitroglycerin may compromise cardiac output (63). However, output improves, $MVO_2$ decreases, and ST elevation regresses because of decreased afterload when failure is present (64). It is apparent that beneficial effects depend primarily on peripheral vasodilatation since intracoronary administration is ineffective (65).

FUROSEMIDE AND ETHACRYNIC ACID Many of the immediate cardiovascular effects of these diuretics are mediated by altered preload resulting from systemic and pulmonary venodilatation rather than diuresis (66). For example, in patients with left ventricular failure furosemide decreased LV filling pressure and increased venous capacitance within 15 minutes, well before any diuretic effect was evident. Unfortunately, diuresis may complicate recovery when these agents are used injudiciously and without adequate hemodynamic monitoring (Table 1).

REDUCTION OF AFTERLOAD WITH CIRCULATORY ASSIST DEVICES Intraaortic balloon counterpulsation augments diastolic aortic pressure, and decreases ventricular afterload by augmenting runoff into the dilated peripheral vasculature during systole.

Counterpulsation in experimental animals improves hemodynamics (67, 68) and decreases ST-segment elevation (69). Left ventricular wall stress and contractile element work decrease by approximately 25% (70). Although the development of morphological criteria of necrosis is delayed, the ultimate extent of infarction, estimated from myocardial CPK depletion (71), is not diminished.

In patients, the incidence of ventricular tachycardia and fibrillation decreases (72), but overall coronary flow is not substantially increased (73). Necrosis is less marked in patients who succumb with cardiogenic shock after balloon counterpulsation than in those with other forms of medical treatment (74). Hemodynamics improve (75), but increased long-term survival has not been observed consistently (76). Accordingly, counterpulsation may be most useful in stabilizing patients hemodynamically prior to infarctectomy and emergency coronary bypass surgery, or prior to surgery for catastrophic complications of infarction (77).

External circulatory assist devices apply pressure to the lower limbs during diastole and promote increased runoff during systole. In contrast to intraaortic counterpulsation, preload may decrease because of constraints on venous return (78). It is not yet clear whether prognosis is improved consistently (79). External counterpulsation decreases the number of PVCs early after the onset of myocardial infarction, but the effect is transitory and not associated with decreased enzymatically estimated infarct size (80).

### *Augmentation of Myocardial Oxygen Supply*

Oxygen is administered almost routinely to patients with myocardial infarction. Although cardiac output is improved in patients with hypoxemia (81), oxygen may produce disadvantageous peripheral vasoconstriction when PAo is normal (82). Thus, even though most patients with myocardial infarction exhibit hypoxemia, arterial desaturation on room air should be documented before continued administration of oxygen is utilized.

In experimental animals with infarction, hyperbaric oxygen increased mean arterial pressure, stroke work, and left ventricular end diastolic pressure, and decreased myocardial lactate production (83). Forty percent compared to 20% oxygen at atmospheric pressure decreased ST-segment elevation after coronary occlusion persisting for 24 hours (84). Hyperbaric oxygen may decrease early mortality in patients slightly (85), but data are inconclusive.

Augmentation of myocardial oxygen supply can be best achieved a priori by myocardial reperfusion. Reperfusion initiated three hours after experimental coronary occlusion preserved myocardium (86), but reperfusion after five hours produced disparate results. Fifty-six percent of conscious dogs exhibited the anticipated salvage of myocardium, but 44% exhibited frank extension of infarction (35). Extension was associated with myocardial hemorrhage, apparently due to insults to vessels in the ischemic zone. Comparable deleterious effects have been observed in patients as well as in experimental animals (87, 88).

The high mortality rate accompanying bypass surgery for acute myocardial infarction may be due to a related phenomenon (89). Only on rare occasions is surgical intervention feasible so rapidly after the onset of infarction that net preservation of myocardium can be anticipated. One example is the occurrence of infarction during cardiac catheterization (90).

Administration of mannitol is another means by which coronary flow has been enhanced experimentally, presumably by means of decreased cell swelling and consequent reduction of the "no reflow" phenomenon. Under defined conditions, protection of ischemic myocardium is demonstrable (91, 92), although this therapeutic approach has not yet been widely evaluated clinically.

### *Metabolic Interventions*

Energy production in hypoxic myocardium depends on glycolysis. Thus, after experimental infarction, hypoglycemia increases ST-segment elevation, myocardial CPK depletion, and necrosis (93). Conversely, intraveneous administration of *glucose-insulin-potassium* (GIK) decreases all three (94).

In patients, GIK decreased the incidence of arrhythmia but did not change the overall mortality (95). Although administration of GIK immediately after the onset of infarction decreased peak serum CPK values in retrospective studies (9), administration of GIK beginning several hours after the initial serum CPK elevation did not preserve myocardium, judging from serial serum CPK changes (96).

*Hyaluronidase,* presumably capable of facilitating diffusion of substrate in ischemic myocardium, reduced epicardial ST-segment elevation, myocardial CPK depletion, and microscopic criteria of infarction in animals (97). In patients doses of 500 μg/kg every 6 hours for 48 hours led to reduced ST-segment elevation (10) and diminished evolution of Q waves (E. Braunwald, personal communication).

Since activation of lysosomal hydrolases may contribute to myocardial injury (11), *corticosteroids* have been used to protect the ischemic heart (98). Hydrocortisone administered to dogs 30 minutes after coronary occlusion decreased myocardial CPK depletion and necrosis (99).

Decreased early mortality has been reported in patients treated with methylprednisolone compared to non-randomized controls studied retrospectively (100), and high doses administered seven hours after the initial serum CPK elevation allegedly protected myocardium (101). However, in other studies administration of methylprednisolone in doses of 30 mg/kg intravenously every six hours for 48 hours was clearly deleterious. Increases in enzymatically estimated infarct size, malignant ventricular dysrhythmia, ventricular rupture, early mortality, and persistent elevation of MB CPK were observed (102). Impaired healing with diminution of scar formation may be responsible for an increased incidence of myocardial rupture (103).

Despite intensive study, sufficient data are not yet available to justify routine clinical use of these or other specific metabolic interventions. This may in part reflect difficulties inherent in methods available for evaluation of their efficacy in patients. For example, studies designed to compare observed to predicted infarct size based on serial CPK changes entail a prolonged interval prior to implementation of the intervention that may contribute to apparent lack of efficacy. Nevertheless, on the basis of experimental findings, it seems likely that specific metabolic interventions will ultimately prove useful in preserving ischemic myocardium in selected patients with acute myocardial infarction.

## MANAGEMENT OF DYSRHYTHMIA

Dysrhythmias associated with infarction require treatment when they precipitate hemodynamic impairment; compromise myocardial viability by increasing $MVO_2$; or predispose or progress to malignant dysrhythmias, including ventricular fibrillation or asystole. Recent evidence suggests that both the diminished threshold to ventricular fibrillation (104) and the incidence of malignant ventricular dysrhythmia (105) are related to infarct size.

### *A-V Block*

Block proximal to the His bundle is often transitory (resolving within several days), associated with inferior myocardial infarction, and readily managed with temporary pacemaker therapy. Infra-His bundle trifascicular block is usually persistent, seen with anterior infarction, and associated with high mortality, probably because the underlying infarction is so extensive. The incidence of sudden death in survivors is substantial and justifies permanent pacemaker therapy (106). In patients with acute myocardial infarction, watchful waiting is indicated when newly developing block is first or second degree, proximal, associated with narrow QRS complexes, demonstrably bifascicular (without P-R or H-V prolongation) (107), or right or left bundle branch block alone (108). However, trifascicular, or progressive block, known to be associated with a high overall mortality (109–112), should be treated with temporary pacing to improve hemodynamics or reduce ventricular irritability, since pacemaker therapy may be lifesaving in a small subset of patients.

### *Bradycardia*

Hypotension associated with sinus bradycardia, or bradycardia due to A-V block, within minutes after the onset of myocardial infarction often responds to atropine; this is the acknowledged treatment of choice (113). However, bradycardia occurring later may have different implications, particularly when hypotension is not present. In open-chest dogs with coronary occlusion, vagal tone and bradycardia increase the threshold to ventricular fibrillation (114). Furthermore, acceleration of heart rate increases infarct size in experimental animals (46). Thus, bradycardia after the initial phase of infarction may not require treatment, and treatment in the absence of hypotension may be deleterious.

### *Ventricular Dysrhythmia*

PVCs occur in most patients with acute myocardial infarction. Contrary to earlier expectations, ventricular tachycardia appears to accompany late, as often or more frequently than early PVCs (115) (G.C. Oliver and R. E. Kleiger, personal communication). Similarly, ventricular tachycardia can be induced most readily experimentally by paced beats with long coupling intervals (116).

Suppression of PVCs and reduction of pre-hospital ventricular fibrillation results from intramuscular administration of lidocaine sufficient to produce blood levels of 2 μg/ml for one hour (117). In the CCU, lidocaine (118), quinidine (119), or procainamide hydrochloride (120) reduce the incidence of ventricular fibrillation substantially. However, because PVCs and other premonitory dysrhythmias as well as ventricular fibrillation itself can be treated (with lidocaine and other antiarrhythmics) so successfully in the CCU, prophylaxis with lidocaine does not lower mortality demonstrably.

Because lidocaine depresses cardiac performance only minimally (121), exhibits few side effects, and is readily titratable, it has become the established treatment of choice for PVCs and ventricular tachyarrhythmias in the CCU. PVCs resistant to suppression with lidocaine occur with high prevailing heart rates (122), and may respond after heart rate has been reduced. Investigational antiarrhythmic agents such as QX 572, a lidocaine derivative, may ultimately prove particularly useful in suppressing lidocaine-resistant PVCs (123). Other agents (investigational in the United States) such as Verapamil, capable of blocking calcium transport across the cell membrane and inhibiting slow current automaticity are likely to be effective antiarrhythmic agents in patients with infarction (124).

Bretylium tosylate (investigational in the United States) sometimes suppresses refractory PVCs. The early catecholamine release induced by the drug may be a useful attribute in patients with left ventricular failure or A-V block (125).

When life-threatening ventricular dysrhythmias persist, overdrive suppression with ventricular pacing (126) or excision of a region of the ventricle responsible for re-entry (127) has occasionally been useful. However, these approaches are rarely indicated in patients with acute myocardial infarction. Recently, β-adrenergic blocking agents have been used to treat ventricular as well as supraventricular tachyarrhythmias associated with acute myocardial infarction, particularly when left ventricular failure is not prominent. For example, Acebutolol, a β-blocker with less of the negative inotropic effects seen with propranolol, suppressed PVCs and decreased the incidence of ventricular fibrillation (128).

When tachyarrhythmias persist and result in impaired ventricular performance, electrical cardioversion is usually required. If digitalis intoxication is suspected, cardioversion of many supraventricular tachycardias (but not atrial fibrillation) may be accomplished by rapid atrial pacing (129).

## MANAGEMENT OF OTHER LIFE-THREATENING COMPLICATIONS

Three catastrophic complications of myocardial infarction require surgical intervention: rupture of the ventricular free wall, the interventricular septum, or a papillary muscle. Intraaortic balloon counterpulsation may provide hemodynamic stabilization prior to immediate surgery or permit its delay until some healing has occurred (130).

Cardiac rupture occurs with extensive infarction which is not confined to the inferior left ventricular wall. Its incidence is approximately 5% (131) and its prognosis is dismal (132). Abrupt bradycardia or electromechanical dissociation may herald its occurrence (133).

Rupture of the interventricular septum occurs in approximately 1% of patients with anteroseptal infarction with a high mortality in the first hour (134). Surgery may be successful when hemodynamic support is sufficient to allow fibrous healing for three weeks prior to operation (135). A similar approach is often required to deal with papillary muscle rupture.

## ANTICOAGULATION AND FIBRINOLYTIC THERAPY

Improved survival has not been observed consistently after fibrinolytic therapy (136–139), but sporadic reports of favorable effects of anticoagulants on mortality continue to appear (140), despite the absence of conclusive findings after more than two decades of evaluation. Although salutary effects on the underlying coronary disease, progression or recurrence of infarction, and long-term survival have not been demonstrable consistently, it is clear that anticoagulation decreases the incidence of cerebral emboli after myocardial infarction from approximately 4 to 1% (141). Furthermore, mini-dose heparin reduces the incidence of deep vein thrombosis substantially (142, 143).

Recently, the possible role of platelets (144) and altered blood viscosity (145) in mediating or potentiating coronary artery disease has been considered. However, the value of anti-platelet drugs such as aspirin or dipyridamole to patients with acute infarction remains conjectural.

## SUMMARY AND GUIDELINES FOR PATIENT MANAGEMENT

As with many medical emergencies, initial evaluation of the patient must be rapid and treatment must often be initiated prior to definitive diagnosis. Infarction is usually confirmed electrocardiographically and by serial changes in plasma enzymes. Detection of elevated MB CPK may be definitive, particularly in patients with other enzymes released from non-cardiac sources (146, 147). Scintigraphy is useful when hospital admission is delayed and elevated enzyme activity may have regressed.

In the Coronary Care Unit, prophylactic administration of antiarrhythmic drugs is not necessary, since conscientious manual monitoring or computer monitoring (capable of detecting more than 99% of PVCs (148), coupled with aggressive treatment of premonitory dysrhythmias and prompt defibrillation, can reduce mortality as effectively. Derangements underlying dysrhythmias such as hypoxemia; alkalosis or acidosis; altered plasma potassium, calcium, or magnesium; digitalis toxicity; or hypovolemia should be identified and corrected. Sinus tachycardia not due to hypovolemia, pericarditis, or congestive heart failure may respond to reduction of anxiety, mild sedation, effective analgesia with morphine, or $\beta$-adrenergic blockade. Sinus bradycardia associated with hypotension in the early phase of myocardial infarction should be treated with atropine. Newly developed trifascicular block or hemodynamically compromising bradycardia due to persistent A-V block often can be best managed with a temporary transvenous pacemaker. PVCs should be treated aggressively to reduce the likelihood of ventricular fibrillation. Ventricular fibrillation or sustained ventricular tachycardia with hemodynamic sequelae requires immediate electrical countershock. Asystole requires immediate pacing, usually best achieved with a transthoracic pacemaker.

Hemodynamic monitoring is useful in any patient with complications such as hypotension, tachycardia, or pulmonary edema. Serial determinations of PAo and cardiac output with the use of a Swan-Ganz thermodilution catheter are useful in adjustment of drug and fluid therapy. A guide for management for subsets of patients categorized hemodynamically is summarized in Table 1. The therapeutic approaches outlined are designed to improve hemodynamics without compromising myocardial viability. Although additional interventions such as administration of GIK, hyaluronidase, and mannitol may become established adjuncts, their efficacy in preserving myocardium in the clinical setting has not yet been demonstrated.

Anticoagulation with mini-dose heparin is useful early after the onset of infarction, particularly in patients with left ventricular failure. Discharge from the hospital after one to two weeks is not associated with increased morbidity or mortality unless specific complications persist after the first six days, including newly developed bundle branch block, ventricular dysrhythmia, recurrent chest pain, left ventricular failure, hypotension, or ST-segment elevation (149–151). When infarction is extensive or complicated, prolonged electrocardiographic monitoring and hospitalization for several weeks are generally required.

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# POTENTIAL MICROWAVE INJURIES IN CLINICAL MEDICINE

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## INTRODUCTION

The clinical uses of microwave radiation in treatment and diagnostic procedures are rapidly increasing. The application of microwaves is desirable in many clinical situations due to the unique capability of microwaves to penetrate biological material and produce heating of deep tissues. As with most other therapeutic techniques, care must be exercised in the use of microwaves, and potential harmful effects due to the misuse must be understood and avoided. To aid in the prevention of potential harmful effects, a basic understanding of microwaves and the interaction of microwaves with different tissues is essential. The purpose of this chapter is to provide the user of microwaves in clinical medicine a basic understanding of potential harmful effects and the precautions necessary to prevent injuries due to microwave treatment.

## POTENTIAL MICROWAVE INJURIES

Rather than attempt to discuss specific cases of injury due to clinical or other use of microwave radiation, I present some of the biological effects that have been reported in the literature and precautions which should be taken during medical use of microwave radiation. The first harmful effect is an indirect but very serious one: where microwaves are used, the potential for electromagnetic interference with medical electronics exists (1). Although this fact is relevant to many types of medical instruments, the emphasis here is on cardiac pacemakers (2–4). Cardiac pacemakers can be designed so that they are insensitive to electromagnetic interference. In recent months, manufacturers have been alerted to the problem and have taken corrective measures by increasing shielding and filtering on leads. However, many individuals (an estimated 20,000) are currently wearing pacemakers which are sensitive to microwave frequencies of 918 MHz and 2450 MHz, microwave frequencies designated for medical use. Information on the interference of microwaves with different model pacemakers is presented in the literature (3, 4). Patients wearing pacemakers should not be treated with microwave radiation until information concerning microwave interference with the particular pacemaker is known.

Many studies on the biological effects of microwave radiation have been made and are summarized in the literature (5, 6). Microwave effects are usually classified as either thermal or nonthermal. Thermal effects are those which result from the heating of biological material, and nonthermal effects result from the direct interaction of the electromagnetic field with the biological material. The predominant use of microwave radiation is for therapeutic heating using diathermy techniques. An excellent up-to-date review of the therapeutic application of electromagnetic power has been presented (7). For diathermy to be optimally effective, the temperature of the treated tissue must be brought to therapeutic levels (40–45°C) without burning the intervening tissues (<45°C). This creates a difficult problem for both the diathermy designer and the clinician since in most cases the energy must be transmitted through the skin and a layer of subcutaneous fat, which has little vasculation and cooling capacity, into muscle tissue, which has much more vasculation and cooling capacity. More energy, therefore, must be delivered to the muscle than to the intervening fat layer. For this reason, the 918 MHz diathermy units are superior to the 2450 MHz units because of the greater depth of penetration and minimal fat heating. The type of application and the distance from the applicator to the tissue surface are also important parameters. Guy (7) has shown that the 918 MHz direct-contact applicator is superior to the 2450 MHz C director, not only because it delivers more energy to the muscle relative to the fat, but also because it limits the amount of extraneous radiation in the room resulting from reflections and scattering from the tissue surface. The 2450 MHz C director produced a radiation level of 10 mW/cm$^2$ at 25 cm from the end of the aperture, while the 918 MHz direct-contact aperture source produced less than 10 mW/cm$^2$ at a distance of 8 cm from the edge of the applicator. This characteristic is important in order to minimize exposure of other parts of the patient's body, as well as personnel in the surrounding area, to undesirable radiation.

The organs of the body most susceptible to microwave radiation are the eyes and the testes. The absorbed microwave energy readily produces elevated temperatures in these organs due to the limited amount of blood flow available for removal of heat. The possibility of damage to the eyes is a very serious aspect of microwave radiation. Cataracts have been produced in the eyes of rabbits at 2450 MHz (8–10). The duration of exposure at various power densities required to produce cataracts is shown in Figure 1. It appears that the threshold for acute exposure (continuous exposure for relatively short times) is approximately 150 mW/cm$^2$. Since the power output of a microwave diathermy unit can be as high as 100 W and the area of the C applicator is of the order of 150 cm$^2$, the power density at the applicator can be approximately 670 mW/cm$^2$. This power density is approximately 4 times the threshold level for producing cataracts in the eyes of rabbits. Extreme care must be used to prevent the exposure of the eyes, particularly when diathermy is used around the head to treat neck sprains or tumors, or to speed healing of wounds due to teeth extraction, oral surgery, or accidents.

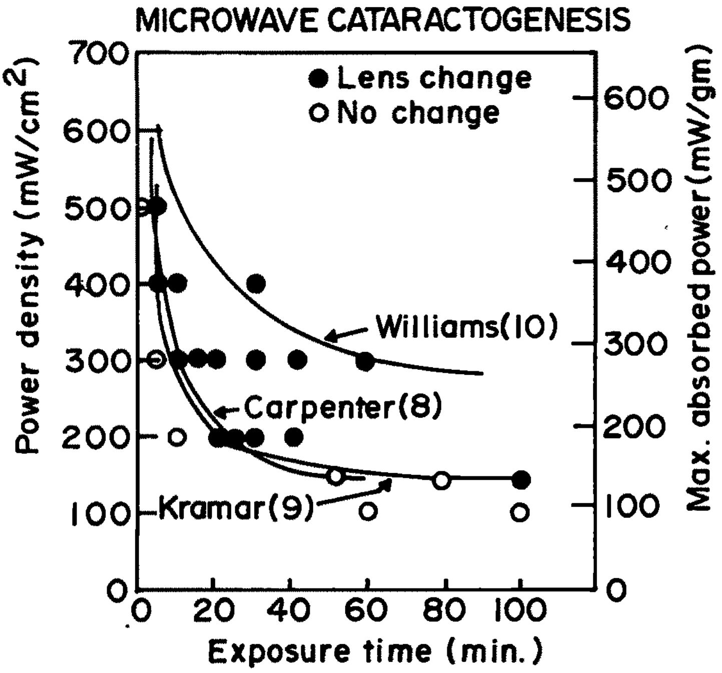

*Figure 1* Power density and exposure time for production of cataracts in the rabbit eye at 2450 MHz.

The testes are extremely sensitive to elevations in temperature. Several investigators have studied the effects of microwaves on the testes (11–13). Imig, Thomson & Hines (11) exposed the testes of male albino rats of the Sprague-Dawley strain to 2450 MHz microwaves and infrared radiation. Testicular degeneration resulted from microwave exposure for 10 min at a temperature of 35°C. Testicular degeneration from 10 min exposure to infrared radiation occurred only when the testes temperature reached 40°C or above. The histological appearance was typical of the degeneration seen in experimental cryptorchism. The types of degeneration resulting from microwave exposure could not be distinguished from those produced by infrared.

Ely, Goldman & Hearon (12) performed a study on dogs, rabbits, and rats in order to determine the threshold for testicular damage. They found that 10 mW/cm$^2$ was the threshold for testicular damage for indefinite exposure. The pathological damage found in the testes include degeneration of the epithelial lining of the seminiferous tubules, and a sharp reduction in the number of maturing spermatocytes in the lumen. The reduction in testicular function due to the heating effect at 10 mW/cm$^2$ appears to be temporary and probably reversible. Haidt & McTighe (13) exposed mice to low level 2450 MHz whole-body microwave radiation (6.5

mW/cm$^2$) for a total of 230 hr over a two month period. No postirradiation increase in rectal temperature was noted. Pathological examination revealed scattered lesions near the surface of the testicles. Within affected tubules, various cellular layers were disorganized and the normal spermatogenic cycle was disrupted. Vacuole formation and giant cells were present in some cases.

The application of microwaves for male contraception is being studied in mice (14). Sperm production has been terminated for up to 8 months. Due to the results reported above and the lack of knowledge about the harmful effects of microwave treatments to males or their progeny, protection of the testicles during microwave treatment should be standard procedure at the present time.

Teratogenic effects due to hyperthermia are well documented in the literature (15). Due to their ability to penetrate to deep tissues without significant surface heating, microwaves can potentially disrupt normal embryonic development when radiation is applied to the abdomen. Van Ummersen (16) has reported inhibition of growth and cell differentiation in chick embryos due to 2450 MHz microwave exposure at 20 mW/cm$^2$ power levels for 280 min. The temperature of the yolk increased 3.5°C above normal incubation temperature during the radiation. In our laboratories, we have exposed fertilized Japanese quail eggs to 30 mW/cm$^2$ for 4 hr during the first five days of development without allowing a temperature increase over the optimum incubation temperature (17). We have also exposed the quail eggs to 5 mW/cm$^2$ for the entire development period of 14 days and an increased temperature of 2°C over optimum incubation temperature. We found no effects on normal development of the embryos, or decrease in hatchability. Carpenter & Livstone (18) have observed that microwave radiation inhibited cell differentiation in mealworm beetle pupae when the temperature increased only 3°C, less than variations in a day-night cycle. Rugh and his colleagues (19) exposed pregnant mice to 2450 MHz for 5 min at an estimated power density of 123 mW/cm$^2$. Anomalies observed in the microwave-irradiated fetuses included gross hemorrhage, resorption, exencephaly, stunting, and fetal death. Exposure of the human female pelvis during early pregnancy and prior to conception has been reported (20). One patient was irradiated during the first 59 days of pregnancy. She aborted on day 67. Three patients were treated with microwaves, conceived within three months after microwave therapy, and delivered normal infants at term. The author stated that microwaves do not appear to interfere with ovulation and conception, but warned against treating pregnant women unless a careful menstrual history is taken. Microwave heating of the uterine wall during parturition has become accepted clinical procedure in some departments (21). The authors caution the user against protracted administration and emphasize that large doses could lead to harmful increases in the temperature of the fetus and the amniotic fluid.

Microwave blood warmers are being used extensively to warm refrigerated blood prior to transfusion. Arens & Leonard (22) have reported three patients who evidenced hemolysis after receiving blood warmed in a microwave unit in spite of having applied recommended precautions. They attribute this result to overheating since no other cause for the hemolysis could be identified. Only specially trained personnel should be allowed to operate blood warmers. Careful measurement of the temperature of the blood and daily calibration using plastic units of saline solution would determine whether or not the device is functioning properly.

The final topic of discussion is the microwave effects on the nervous system and behavior. Many studies in the Soviet literature have reported headaches, increased fatigability, increased irritability, loss of appetite, sweating, difficulties in concentration or memory, depression, emotional instability, and thyroid gland enlargement (23). In the Soviet Union, these effects are regarded as typical microwave-induced disturbances and are referred to as the asthenic syndrome. Clinical manifestations of chronic occupational exposure of humans to microwave radiation reported by Letavet & Gordon (24) include bradycardia, disruption of the endocrine-hormonal process, hypotension, intensification of thyroid gland activity, exhausting influences on the central nervous system, decrease in sensitivity to smell, and increase in histamine content of the blood.

In the United States, Olson and his colleagues (25) have shown that bradycardia occurs in isolated rat heart preparations when exposed to low level 960 MHz microwave radiation (1.5–2.5 $mW/cm^3$ absorbed power). The heart rate decreased to a value of 15–40% below preirradiation rates, and in some experiments temporary cessations of the heart contractions lasting 5–12 sec occurred. Frey (26) has found that the perception of sound can be induced in humans irradiated with modulated microwave radiation. Frey has also detected evoked electrical potentials in the brain stem of cats as a result of radiating with pulsed-modulated microwave fields. Bawin and her colleagues (27) have exposed cats to a 147 MHz electric field, amplitude modulated at the biological frequencies (1–25 Hz). The experimental results showed that the fields increased the rate of occurrence of the spontaneous rhythms when the microwaves were modulated at frequencies close to the biological dominant frequency of the selected intrinsic EEG rhythmic episodes. The irradiated group also differed from the control group in the rate of performance, accuracy, and retention of learned tasks. Additional research into the neurological and behavioral effects of microwave radiation is required before the consequences of such exposure can be specified with certainty.

## CONCLUSION

Microwaves have unique capabilities that are useful in clinical medicine. As with many other therapeutic procedures, the potential for microwave injury exists when improper or careless use of this capability occurs. The thermal effects of microwave radiation are adequately understood, since the hyperthemia induced in tissue by microwaves has the same relative effects as other heating techniques. However, the ability to deliver the desired amount of microwave energy to the specific location without overheating organs, embryos, or other thermal-sensitive tissues requires a knowledge of both the equipment being utilized and the properties of the microwave radiation. Persons trained in the use of microwave equipment should be available when microwaves are administered.

A second concern when using microwave medical equipment is the exposure of portions of the patient's body not being treated and operators of the equipment to microwaves as a result of scattering and reflection from the tissue surface. The biological effects of low level chronic exposure to microwave radiation are not well known. The accepted safe level of microwave exposure of the general population as established by the American National Standards Institute is 10 mW/cm$^2$. Precautions should be taken to prevent exposure of the operator and others in the surrounding area by using the equipment in properly shielded rooms. Microwaves should be used judiciously in clinical medicine due to their potential for causing injury despite their therapeutic value.

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# GLOMERULAR INJURY IN PATIENTS WITH NEOPLASIA

❖7186

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## INTRODUCTION

The intriguing possibility of an association between the nephrotic syndrome and malignancy was raised by Lee et al (1) in 1966 and alluded to in sporadic case reports prior to that time. However, definitive evidence linking these conditions has only been forthcoming recently with the demonstration of tumor-derived antigens on the glomerular basement membranes of patients with glomerular injury and extrarenal malignancies (2–4).

A variety of malignant and some benign tumors have been associated with many different pathologic forms of glomerular injury and the implication in this review is that the tumors play a role in the pathogenesis of the glomerulopathy. However, in some cases the association may have been fortuitous, or the tumor may have developed as a complication of uremia (5).

## NEOPLASIA AND RENAL DISEASE

There are several ways in which renal disease can occur as a result of an extrarenal malignancy (6). Direct consequences of the tumor may include metastatic invasion of renal parenchyma, hydronephrosis from retroperitoneal adenopathy or retroperitoneal fibrosis, and renal vein thrombosis. Although renal vein thrombosis has been considered a cause of the nephrotic syndrome (7), it may also be a consequence thereof (8–10) and can be a complication of malignancy. Renal amyloidosis may also be a complication of malignancy and can cause the nephrotic syndrome. Complications of malignancy such as hypercalcemia can induce a nephropathy, as can complications of therapy such as hyperuricemia. The kidneys can also be damaged by irradiation and during treatment with iphosphamide (11). Tubular obstruction due to Bence-Jones protein occurs in patients with myeloma and has occurred as a result of mucoproteins in a patient with a pancreatic adenocarcinoma (12). As reviewed in this paper, neoplasms can also induce glomerular injury, usually accompanied by the nephrotic syndrome.

### *Criteria for an Association Between Glomerular Injury and Neoplasia*

It is important to categorize the levels of evidence that have been used to indicate a causal association between neoplasia and glomerular injury. The most incontrovertible evidence is the demonstration of an antigen on the glomerular basement membrane, which is shown to be derived from the patient's tumor. Isolation of an etiologic agent such as a virus that could cause both the tumor and the glomerulopathy would also be important evidence. However, since a tumor-derived antigen has rarely been demonstrated, most of the cases have been accepted on circumstantial evidence. This includes remission of the nephrotic syndrome with treatment of the tumor, relapse of the renal disease with recurrence of the malignancy, and a temporal association between the diagnosis of glomerulonephropathy and malignancy. Furthermore, in many of the published cases other causes of the nephrotic syndrome such as renal amyloidosis, systemic lupus, diabetes mellitus, cryoglobulinemia, and syphilis were eliminated. Although an increased prevalence of $\beta$-hemolytic streptococcal infection has been noted in patients with neoplasia (13), evidence of an antecedent streptococcal infection has not been found in patients with malignancy and glomerular injury (14).

### *Prevalence*

Precise information on the prevalence of this association is lacking. Lee et al (1) found 11 neoplasms in 101 patients with the nephrotic syndrome (10.9%). This was greater than the expected incidence of neoplasia in the age group of their nephrotic patients. More recently, Hopper (10) has reported a 6% prevalence of cancer in 82 patients with membranous glomerulopathy.

In an attempt to obtain an estimate of the prevalence of this association, we reviewed 14 series comprising 1643 patients with the nephrotic syndrome and found that a malignancy was mentioned in only six cases. However, we are convinced that failure to appreciate the significance of a tumor in patients with glomerular injury resulted in this very low figure.

## CARCINOMA AND GLOMERULOPATHY

More than 30 patients have been reported in whom carcinoma was associated with the nephrotic syndrome, although in some the association was tenuous. Until 1966 there were very few reports (15, 16). Lee et al (1) then described 11 cases and suggested that there might be an etiologic relationship, possibly on the basis of a perturbation in the patient's immunologic mechanisms. In these cases the onset of the nephrotic syndrome occurred from 14 months before to one month after the diagnosis of a carcinoma. Eight patients died from the tumor and one of renal failure.

Although carcinomas originate from many different organs (1), the two most common sites are the lung and colon. Remission of the nephrotic syndrome has been described following excision of carcinomas in at least three patients (1, 10, 17).

There is strong evidence in four patients that the glomerular lesion was related to an immune response to a tumor-associated antigen with deposition of antigen-antibody complexes in relation to the glomerular capillary membranes. Lewis et al (18) studied a patient with bronchial carcinoma and membranous glomerulonephritis. Elution of the immunoglobulins present in a granular distribution along the glomerular basement membrane revealed IgG and IgA reacting specifically with plasma membrane antigens of the bronchial tumor cells. Costanza et al (2) demonstrated carcinoembryonic antigen (CEA) along the glomerular basement membrane of a patient with carcinoma of the colon, in the same distribution as deposits of IgG, IgA, IgM, and $C_3$ globulin. Couser et al (3) studied a 60-year-old man with membranous glomerulopathy and colonic carcinoma in whom circulating CEA was detected. They found a tumor antigen along the glomerular basement membrane which had similar biologic properties to CEA but was unrelated to it immunologically, and an antibody in the serum which was reactive with an antigen deposited on the glomerular basement membrane. A fourth patient had malignant melanoma, hypocomplementemia, the nephrotic syndrome, and melanoma antigen and antibodies on the glomerular basement membrane (4).

Details of renal histology have been available in at least 26 cases [including six of our cases (14)]. Of considerable interest is the predominance of membranous glomerulonephritis in patients with carcinoma, in contrast to patients with Hodgkin's disease in whom the renal abnormality is usually the minimal lesion form of the nephrotic syndrome. Sixty percent of patients (16 of 26) with carcinoma and the nephrotic syndrome had a membranous glomerulopathy and 30% (8 of 26) had increased mesangial matrix and cells (14). Only one patient had the minimal lesion nephrotic syndrome.

## HODGKIN'S DISEASE

Hodgkin's disease has been the single most common malignancy reported in association with the nephrotic syndrome. Although a definite link has not been established, there is circumstantial evidence supporting this relationship: Hodgkin's disease and the nephrotic syndrome may present at the same time, they may relapse together, and treatment of the malignancy is usually followed by a remission of the renal disease.

At least 30 cases with this association have been reported (19–28) and Plager & Stutzman (22) discussed the clinical and pathologic features of 14 patients who ranged in age from 2 to 62 years, with a male to female ratio of 2:1. Many of the patients had gross proteinuria with levels up to 30 g/24 hours. All had hypoalbuminemia and most had hypercholesterolemia. The blood urea nitrogen and/or serum creatinine concentrations were only occasionally elevated. Hematuria was the exception and the serum $C_3$ concentrations were normal. Hypertension and uremia were infrequent complications and death due to renal failure was uncommon.

The clinical features of the Hodgkin's disease presented before or at the same time as those of the kidney disease in 23 patients; however, in seven cases a diagnosis of the nephrotic syndrome was made prior to that of the malignancy (21–25, 27, 28). Remission of the nephrotic syndrome followed successful treatment of the Hodgkin's disease at least once in 26 of 28 patients and occurred after radiation therapy, alkylating agents, steroid therapy, surgical excision of a localized tumor, and combined therapy with nitrogen mustard, vincristine, and procarbazine. Of particular interest were patients in whom the renal disease remitted after therapy of localized disease, such as surgical resection of a tumor in the neck or after irradiation of the neck, axilla, and mediastinum (22).

Kidney tissue was studied by light microscopy in 24 cases, by immunofluorescent microscopy in five, and by electron microscopy in 11. In 17 cases light microscopy revealed glomeruli which were normal or had a mild degree of mesangial cell proliferation. Three patients had thickened glomerular basement membranes and two had a proliferative glomerulonephritis. Immunoglobulin deposits were found in three of five cases studied by immunofluorescent microscopy. In five cases, either subendothelial (27), intramembranous (26), or subepithelial deposits (25, 28) have been noted.

Although six patients had remissions and relapses of the nephrotic syndrome, progression of the histologic lesion from the minimal lesion nephrotic syndrome to focal glomerulosclerosis was reported in only one patient (27).

## CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

There are reports of six patients aged 49 to 64 years (five men and one woman) with CLL and the nephrotic syndrome (29–33). Both conditions were diagnosed at about the same time in four and the nephrotic syndrome presented 2 and 4 years after the diagnosis of CLL in two patients. Improvement in both the nephrotic syndrome and CLL was documented in two cases (30, 32).

Renal tissue was studied in five cases: two had IgG and $C_3$ deposits along the glomerular capillary walls and one each had membranous glomerulonephritis, "sclerosing proliferative glomerulonephritis" and "membranoproliferative glomerulonephritis."

## LYMPHOSARCOMA

Three patients have been reported with lymphosarcoma and the nephrotic syndrome (15, 34, 35). In two, the lymphosarcoma manifested 8 and 15 months after the presentation of the renal disease. The nephrotic syndrome and rapid deterioration in renal function occurred two years after diagnosis of a lymphosarcoma in a man who had no evidence of renal disease when the malignancy was diagnosed. He had a membranoproliferative glomerulonephritis with IgG and $C_3$ globulin deposition along the glomerular capillary walls. Electron microscope studies demonstrated large accumulations of electron-dense material along the subendothelial side of the glomerular basement membrane and in the mesangium (14). The other two patients with lymphosarcoma had a membranous glomerulonephritis.

The renal histology in patients with chronic lymphocytic leukemia, lymphosarcoma, lymphocytic lymphoma (36), reticulum cell sarcoma (37), and Burkitt's lymphoma (27) contrasts markedly with that reported in patients with Hodgkin's disease. In 13 of the first group of patients a proliferative glomerulonephritis was seen in seven and a membranous glomerulopathy in five, whereas with Hodgkin's disease, most of the cases had the minimal lesion nephrotic syndrome.

## WALDENSTROM'S MACROGLOBULINEMIA

Passive deposition of IgM was considered an explanation for the large subendothelial deposits of this immunoglobulin in patients with Waldenstrom's macroglobulinemia and glomerulopathy (38). However, recent reports implicate immunologically mediated mechanisms in the pathogenesis of the glomerulonephritis. A patient with Waldenstrom's macroglobulinemia, angiitis, and myositis had a mesangiocapillary glomerulonephritis (39). Electron microscopy revealed electron-dense deposits in the glomerular basement membrane and immunofluorescent microscopic studies showed $C_3$ globulin, IgM, and IgA. A second patient with Waldenstrom's macroglobulinemia and nephrotic syndrome had no glomerular abnormalities disclosed by light microscopy; electron microscopy revealed electron-dense deposits in the glomerular basement membrane and immunofluorescence showed deposits of $C_3$, IgM, and IgG. The nephrotic syndrome improved after treatment with corticosteroids and chlorambucil (40).

## WILM'S TUMOR, PSEUDOHERMAPHRODITISM, AND GLOMERULAR INJURY

Most cases of malignancy and glomerulopathy occur in older patients. However, in young infants there is an association encompassing Wilm's tumor, genital abnormalities, and glomerular injury (41). The occurrence of this constellation of abnormalities suggests a teratogenic etiology for the renal disease. This is partially supported by the fact that many of these infants have had renal histologic features of the congenital nephrotic syndrome (Finnish type) (41).

## BENIGN TUMORS

Attention has focused mainly on the association between malignant neoplasms and glomerular injury. However, there have been a few reports suggesting that benign tumors may also induce the nephrotic syndrome (42, 43). Remission of the nephrotic syndrome occurred in a girl following excision of a carotid body tumor (42), and a similar experience has been noted by Cosby et al (43) in three patients with benign tumors.

## PATHOGENESIS OF RENAL LESIONS IN MALIGNANCY

The most convincing experimental evidence for an association between malignancy and glomerular injury has been obtained from studies in mice. New Zealand mice frequently develop a lupus-like syndrome (44) with antinuclear antibodies, hemolytic anemia, and glomerulonephritis; many have lymphomas, chronic infection with murine leukemia virus (MuLV), and circulating MuLV-antiviral antibody complexes. An immune complex form of glomerulonephritis was suggested by granular deposits of immunoglobulin and complement along the glomerular capillary walls and in the mesangium. This was confirmed by the demonstration in the deposits of double-stranded DNA and MuLV antigens, and by elution from the glomeruli of antibodies to the envelope glycoprotein gp 69–71, reverse transcriptase Mu gs-1, RNA, RNA-DNA hybrids, and single- and double-stranded DNA (45, 46). Forms of immune complex glomerulonephritis occur in mice infected with Maloney sarcoma virus, and the polyoma, Raucher, Friend, and Gross oncogenic viruses (47). Non-oncogenic viruses can cause immune complex glomerulonephritis in mice, hogs, horses, and man (47). Furthermore, glomerulonephritis has also been reported in mice and cats with leukemia (48, 49) and in dogs with many different types of neoplasia (50).

Immunohistochemical studies of renal lesions in many patients with malignancy have suggested an immune complex pathogenesis for the glomerular injury. Dixon (51) demonstrated circulating immune complexes in 20 of 48 patients with carcinomas and in 20 of 46 with leukemias, lymphomas, and sarcomas. The interspecies antigen gs-3 of mammalian oncornavirus was found in the glomerular deposits of two patients with acute myelocytic leukemia (52). Carcinoembryonic antigen was demonstrated in the glomerular deposits of a patient with colonic carcinoma (2), and tumor antigens were found in the glomeruli of patients with bronchogenic carcinoma (19), colonic carcinoma (4); and malignant melanoma (3). Studies in a patient with oat cell carcinoma of the lung and the nephrotic syndrome (53) suggested that antibodies formed against nuclear antigen from the necrotic tumor. The resulting complexes localized in the kidney and produced a membranous glomerulonephritis.

Thus, although there is good evidence that circulating immune complexes can induce glomerular injury in patients with tumors, it is of interest that a similar incidence of subclinical glomerulonephritis has been found in patients with malignancies as in autopsied cases who did not have tumors (54).

In Hodgkin's disease, immunohistopathologic studies have not revealed evidence of an immune complex pathogenesis of the nephrotic syndrome (22). However, many similarities have been noted between Hodgkin's disease and minimal lesion nephrotic syndrome, such as male predominance, response to steroids and alkylating agents, prolonged therapeutic remissions, minimal change glomerular morphology, and remissions following measles (55). This has led to the speculation that these conditions may be due to a systemic defect in T-cell function with secretion of a lymphokine which increases the permeability of the glomerular basement membrane to protein (55).

## SUMMARY

There is considerable circumstantial evidence relating neoplasia to glomerular injury. Recently, more convincing evidence has been derived from the demonstration of tumor-associated antigen or antibody to such antigen, in relation to glomerular basement membranes in four patients with glomerular injury and cancer. The most common form of glomerulopathy reported in patients with carcinoma has been membranous glomerulonephritis. However, increased mesangial cells and matrix have also been found in some patients with hematuria and progressive renal failure. In contrast, most patients with Hodgkin's disease and glomerulopathy have had the minimal lesion-type nephrotic syndrome, which has usually responded to successful treatment of the Hodgkin's disease. Glomerular abnormalities have also been reported with chronic lymphocytic leukemia, lymphosarcoma, Waldenstrom's macroglobulinemia, and benign tumors. When there is no apparent cause, proteinuria with or without hematuria or impaired renal function should suggest the possibility of associated neoplasia, particularly in elderly patients.

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# BIOLOGY OF ENDOTOXIN

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## INTRODUCTION

The incidence of and mortality from gram-negative bacterial sepsis is increasing (1). The morbidity and mortality associated with infections caused by gram-negative bacteria are considered to be related to the endotoxins present in the cell walls of these bacteria. Endotoxin-like materials have been isolated from other organisms; however, this review is concerned only with endotoxins from gram-negative bacteria. The chemical and biological properties of bacterial endotoxins have been under investigation for more than a century. During the past two decades more refined chemical methods for the extraction and purification of bacterial endotoxins have been developed. With the production of relatively pure materials, the biological properties have been defined in animal and human experiments. Although endotoxins have a myriad of biological activities that affect all systems of the body, no clear-cut molecular-biological basis for these activities is known. This review focuses on the chemical composition, methods of detection, and some of the biological properties of bacterial endotoxins, with particular emphasis on the effects of these agents in human beings.

## CHEMICAL COMPOSITION

Bacterial endotoxins consist mainly of lipids and polysaccharides with a small amount of amino acids (2). The chemical name lipopolysaccharide (LPS), is used synonymously with bacterial endotoxins. It is possible to divide the endotoxin molecule into three parts: the lipid moiety, the R-core, and the O-polysaccharide.

### *Lipid Moiety*

Acid hydrolysis of LPS produces a phosphorus-containing lipid precipitate, designated as Fraction A (later called Lipid A), and a soluble Fraction B, which contains polysaccharides. The term Lipid A has persisted through the years and refers to all chemical compounds that become insoluble after acid hydrolysis of LPS. Therefore, Lipid A is a mixture of several chemical compounds rather than one chemically defined molecule.

The basic structural component of the lipid in the precipitate is a diglucosamine unit that contains fatty acids attached to amino and hydroxyl groups. Therefore, the lipid component of LPS is composed entirely of fatty acids, which have a range of chain lengths from at least $C_{10}$ to $C_{22}$ (3). The fatty acid usually found in the highest concentration is $\beta$-hydroxymyristic acid, which seems to be unique to the LPS molecule (4). It appears that the glucosamine units are bound together by 1,6-glycosidic linkages and 1,4-phosphodiester bridges (5–7). The LPS molecule which is the outermost part of the cell wall of the gram-negative bacterium is attached via the lipid moiety.

### *R-core*

The R-core is the middle portion of the LPS molecule, linking the lipid moiety to the O-polysaccharide moiety. Investigation of the structure of the R-core has been greatly facilitated by a group of mutant bacteria termed R or rough forms, which are devoid of O-polysaccharide. These bacteria are easily identified by their rough colonial morphology, in contrast to the S or smooth forms, which contain the O-polysaccharide moiety. Chemical analyses of the R-core have shown five basal sugars, phosphate, and O-phosphorylethanolamine (8).

### *O-Polysaccharide*

The O-polysaccharide structure determines the O-antigenic specificity of the bacterium. Biochemical analyses of the O-polysaccharide have established a regular sequence of the sugars (9). The repeating sequences may either be linear or may contain one or more side branches of the component sugars. The length and the composition of these repeating units vary from strain to strain (10).

## ASSAY METHODS FOR LPS

Up until the last decade, assay procedures for LPS in tissues and body fluids were based upon the toxicity of LPS in vivo, i.e. the ability of LPS to produce fever and dermal necrosis in rabbits or death in adrenalectomized mice or chick embryos. Recently, an in vitro method of assay for LPS has been introduced (11). The use of *Limulus* amebocyte lysate (LAL) for the detection of LPS is based upon an in vitro interaction between LPS and a lysate prepared from amebocytes, the only circulating blood cell of the *Limulus* crab (12). It has been reported that this method can detect picogram quantities per milliliter of a purified LPS (13). However, since the biological activities of each LPS are not a uniform gravimetric property but vary (14), it may be virtually impossible for any method to compare different LPSs quantitatively with any meaningful accuracy.

Reports of the clinical value of the LAL test for the detection of endotoxemia in man are discordant. Two groups of investigators have found the LAL test to be a clinically useful and reliable method for the detection of endotoxemia (15, 16). Four other groups have concluded that the LAL test has limited clinical utility for the detection of endotoxemia (17–20). These discordant results might be explained by variations in LAL preparation and sensitivity, the method employed to prepare the plasma sample for LAL testing, the patient population, and statistical analyses. On the other hand, the LAL test has been reported to be clinically useful for the detection of gram-negative bacterial meningitis (21) and gram-negative bacteriuria (22). In addition, the LAL test has been found to be useful for routine pyrogen screening of short-lived radiopharmaceuticals and parenteral solutions (23–25).

The specificity of the LAL test to detect only endotoxin has been questioned. The polynucleotide polyriboinosinic-polyribocytidylic acid (26) and peptidoglycan, isolated from the cell walls of various gram-positive organisms (27), will produce gelation of LAL. Additional studies will be needed to define the specificity of this test.

## BIOLOGICAL PROPERTIES OF LPS

### *Fever*

The fever produced by LPS may occur by direct action on the thermoregulatory center in the brain or, more likely, by indirect LPS action on blood leukocytes, on reticuloendothelial cells, or perhaps on other tissues to liberate an endogenous pyrogen (28). The evidence for the direct action of LPS on the brain is based upon studies in which the injection of LPS into the central nervous system resulted in fever from doses of LPS much less than those required if injected intravenously (29). After an intravenous injection of LPS, there is a lag period of 10–20 minutes in the rabbit and of one to two hours in man before temperature elevation begins. The length of the lag period is dose dependent. During this period, LPS is rapidly removed from the blood by phagocytic cells. These cells are activated by LPS to release a fever-producing substance, which has been named *endogenous pyrogen* (30). The activation process requires new protein and RNA synthesis (31). It has been recently discovered that at least two human phagocytic blood cells, granulocytes and monocytes, produce chemically distinct pyrogens (32). These low molecular weight proteins, which are heat labile, then act on the anterior hypothalamus to produce fever (33). The fever is produced by alteration of either vasomotor control of heat redistribution or heat production (34). Man is the most sensitive of all animals studied to the pyrogenic effects of endotoxin on a weight for weight basis (35).

The repeated administration of LPS to man or experimental animals results in a progressive diminution of some of the biological effects, especially fever. The precise mechanism of this phenomenon, termed *endotoxin tolerance*, is unknown. Early experiments suggested that enhanced activity of the reticuloendothelial system (RES) was responsible for producing tolerance (36). Other studies have shown that endotoxin tolerance persists following RES blockade in animals (37), that tolerance develops in man without a change in RES activity (38), that passive transfer of tolerance in rabbits readily occurs (39), and that experimental stimulation of the RES with zymosan or BCG (bacille Calmette Guérin) fails to induce a state of tolerance (40). Other studies suggest that a 19S γ-globulin fraction of serum is responsible (41), although O antibodies are clearly not the cause of endotoxin tolerance (42). It may also be that tolerance is an inability of the Kupffer cells of the liver to produce endogenous pyrogen (43).

Although the refractory state known as endotoxin tolerance has received considerable attention in the laboratory, its relevance to human disease remains a mystery. For example, the induction of tolerance to *Salmonella typhosa* endotoxin does not alter the acquisition of typhoid fever in human volunteers (44). Furthermore, following acute typhoid fever, humans are tolerant to *S. typhosa* endotoxin (45), despite the fact that during the acute phase of the disease they are hyperreactive to LPS (44). A diverse array of human diseases, such as pyelonephritis (46) and malaria (47), produce endotoxin tolerance, while another, brucellosis, results in hypersensitivity to LPS from brucella organisms following the acute disease (48). Thus, the precise role of this state in the clinical picture of resistance to or recovery from infectious diseases in man is unclear.

## *Effects on Blood*

LEUKOCYTES The intravenous administration of LPS to humans and experimental animals produces transient leukopenia followed by marked granulocytosis. The degree and length of the leukopenia are dose dependent. The leukopenia has been shown to result predominantly from a decrease in granulocytes, due to an increased margination of circulating granulocytes at the endothelium of capillaries (49). The granulocytosis seen after LPS stimulation is dependent on an adequate bone marrow reserve of granulocytes and on a normal granulocyte-releasing mechanism (50). These findings have been used clinically for the estimation of bone-marrow granulocyte reserves and are a useful adjunct to cytotoxic chemotherapy (51), studies of various neutropenic states (52), and regulation of granulocyte production (53). It appears that the production of a granulocyte-releasing factor is increased by LPS injection (54). In addition, LPS injection increases the production of a colony-stimulating factor, which may regulate granulocytopoiesis (55). The development of tolerance to the effects of endotoxin has been shown for both of these factors in mice (56) but not in man (57). In vitro studies have shown that LPS in the presence of complement is ingested by, or adheres to, the human granulocyte, and induces a series of metabolic changes resembling those associated with phagocytosis (58).

ERYTHROCYTES Studies in mice have shown that a single injection of LPS results in a significant shift in erythropoiesis from the bone marrow to the spleen (59). The leukocytic hyperplasia in the bone marrow following LPS administration may cause this shift in erythropoiesis (60).

PLATELETS Thrombocytopenia has been observed in experimental animals after the administration of relatively high doses of LPS (61). This thrombocytopenia is probably due to platelet aggregation following LPS injection, which has been demonstrated in vivo in rabbits and rats (62, 63). After aggregation, degranulation of platelets occurs, which results in an increased activity of platelet factor 3 (64).

Electron microscopy has shown that LPS adheres to platelets, causing them to aggregate, fuse, and fragment (65). A platelet-endotoxin interaction seems to be necessary for the final detoxification of intravascular endotoxin by the RES (66).

DISSEMINATED INTRAVASCULAR COAGULATION (DIC) One of the major complications of gram-negative septicemia in man is the frequent occurrence of DIC (67). The DIC is considered to be secondary to the LPS on the bacteria since there is experimental evidence that LPS may initiate DIC by two different pathways. The first, the interaction between platelets and LPS, liberates platelet factor 3, which in the presence of calcium and factor V acts on activated factor X to form blood thromboplastin, which transforms prothrombin to thrombin (64). Second, LPS can activate directly factor XII (Hageman factor), the initial component of the intrinsic clotting system (68). The activation occurs by the formation of a complex between the lipid moiety of LPS and factor XII (68). The importance of the coagulation sequence in LPS-induced DIC and subsequent mortality is unclear (69, 70) since in patients with gram-negative bacteremia and DIC, correction of the DIC with heparin did not decrease the mortality rate (71).

COMPLEMENT LPS has been shown to activate the complement system via the alternate pathway, as demonstrated by the consumption of the terminal complement components (C'3–C'9) and the sparing of the early complement components (C'1, C'4, and C'2) (72, 73). However, recent studies suggest that LPS is capable of activating both the classical and alternate complement pathways (74). The biologically active products generated due to the activation of the complement system by LPS effect changes in the clotting system, in vascular permeability, in smooth-muscle reactivity, and in neutrophil chemotaxis (72). Indeed, several of the biological changes induced by endotoxin may be mediated by activated components of the complement system.

### *Effects on the Vascular System*

The complex interaction of LPS with the cardiovascular system and the production of shock has been intensively investigated since the syndrome of endotoxin shock in man was first described in 1951 (75). The progress (or lack thereof) in the understanding of the pathogenesis and of the hemodynamic and biochemical events of endotoxin shock is indicated by the still appallingly high mortality rate of this condition in man.

The syndrome of endotoxin shock begins with gram-negative septicemia and resultant endotoxemia. The LPS reacts with leukocytes, platelets, the complement system, and other serum proteins to increase blood levels of proteolytic enzymes and of certain vasoactive substances, such as histamine, kinins, and serotonin (76). This results in pooling of blood, primarily in the pulmonary and splanchnic vasculature (77), and increased peripheral vasoconstriction with resultant poor tissue perfusion. Cardiac output is diminished by the direct myocardial depressant action of LPS and by a decrease in venous return secondary to the pooling of blood (78). The decrease in cardiac output initiates a circular series of events, beginning with a drop in blood pressure and peripheral perfusion. Endogenous catecholamines are liberated to compensate for the falling blood pressure; however, the arteriolar-venular constriction produced by the catecholamines leads to tissue acidosis and anoxia (79). This initiates capillary congestion and dilatation, which completes the circle by accentuating the decreased venous return and cardiac output. In time, the cellular hypoxia results in lysosomal disruption, leading to cell lysis, propagation of tissue injury, and death (80). A concomitant feature of this syndrome is DIC. Whether DIC is a cause or an effect of endotoxin shock is unknown, but the localized or generalized hemorrhagic diathesis due to the consumption of clotting factors certainly intensifies the shock condition.

Current therapy for endotoxin shock attempts to disrupt the circle of events mentioned above by restoring and maintaining the integrity of the microcirculation. This treatment regimen begins with massive fluid administration and plasma expanders. Although of unproven benefit, $\beta$-adrenergic stimulators, $\alpha$-adrenergic blockade, and large doses of corticosteroids are frequently administered (81). The DIC can be reversed in most cases with small doses of heparin (82, 83). In man, the most effective therapy is the use of specific antibiotic treatment of the underlying infection, and supportive measures such as adequate fluid replacement, maintenance of an airway, etc.

### *Effects on the Endocrine System*

The reactivity of the hypothalamic-pituitary-adrenal axis to endotoxins is a well-recognized and clinically useful phenomenon (84). LPS has a selective effect on the hypothalamic-pituitary system, causing the release of adrenocorticotropic hormone (ACTH) and growth hormone (GH) but having no influence on thyrotropin (TSH) and luteinizing hormone (LH) release (85, 86). The site of LPS action on the hypothalamic-pituitary axis has not been settled. There is evidence that LPS acts upon or through the neuronal elements of the hypothalamus to cause the release of ACTH by the pituitary gland (87). On the other hand, studies show that LPS can bypass the medial hypothalamus and act directly upon the pituitary gland to release ACTH (88, 89). Also, a significant elevation of plasma cortisol can be demonstrated two hours after LPS injection (90, 91). Fever and associated nonspecific stress do not appear to be the stimuli for the release of ACTH by LPS (90, 92).

LPS is used clinically to evaluate the endocrine system. In investigating pituitary-adrenal function, LPS is administered and plasma cortisol or growth hormone is measured (84, 93). The cortisol response follows a dose-response relationship and is a rapid and convenient way of testing pituitary-adrenal function. Also, the increased release of growth hormone by LPS is useful in the diagnostic evaluation of hypothalamic or pituitary disorders (86). The peak concentration of growth hormone is achieved at two hours (90). It is of interest that considerably more endotoxin must be administered to result in growth hormone release than is needed to elevate blood cortisol levels (90, 92). Furthermore, less endotoxin is required to induce fever in man than to induce either endocrine response and, in addition, granulocytosis can result from doses of endotoxin that are neither pyrogenic nor capable of inducing release of either cortisol or growth hormone (35, 57).

## *Immunological Phenomena*

In 1927, White established that the somatic polysaccharides of LPS on the surface of gram-negative bacteria confer on these organisms their characteristic serologic specificities (94). This was followed in the early 1930s by the pioneer work of Boivin, who extracted and isolated these LPSs and showed that the same complex carried both antigenic and toxic attributes (95). It has now been clearly established that the repeating oligosaccharide units of the O-polysaccharide portion of the LPS molecule represent, in part or completely, the antigenic determinant group (96). The grouping of *Salmonella* by the Kauffmann-White Schema depends on the O-polysaccharide antigen. In general, the O-polysaccharide of LPS is a potent antigen that can elicit antibody formation in animals in submicrogram quantities (97).

The immune response to LPS in all species studied is quite characteristic (98). At three to four days after intravenous injection of LPS, circulating antibodies to that LPS are measurable, reaching their maximum concentration at six to eight days (99). The antibody response occurs after administration of nanogram quantities of LPS in man (99). Although the initial antibody response is predominantly IgM, antibodies of the IgG and IgA classes are present within a few days (99).

Another interesting property of LPS is mitogenicity. The existence of two main lymphocyte populations, thymus derived (T) and thymus independent (B), is well established. The mitogenic response to LPS appears to be carried out by B cells only (100). This response is not mediated by immunologic mechanisms (101).

Extensive studies have unequivocally documented the adjuvant effect of LPS. Adjuvancy has been shown by increase of titers of circulating antibodies, increase in the number of antibodies, increase in the number of antibody-producing cells, accelerated antigen eliminations, shortening of the lag period of the immune response, and/or longer persistence of circulating antibodies (102). This adjuvant effect has been demonstrated in several animal species, including mice (103), rats (104), rabbits (105), guinea pigs (102), and chickens (102). On the other hand, LPS can act as an immunosuppressant by manipulation of the dose and time of injection relative to administration of the antigen (106).

## *Shwartzman Phenomenon*

A local tissue reactivity was described in 1928 by Shwartzman, who observed that if a single injection of a culture filtrate of *Salmonella typhosa* was made into the skin of a rabbit, and if the same material was injected intravenously 24 hours later, a severe hemorrhagic necrotic lesion developed at the site of the original skin injection (107). The reaction was nonspecific, since the filtrate used for the preparatory skin injection showed the same reaction after the intravenous injection of the filtrate from another organism. The component of the culture filtrate that produced the localized Shwartzman reaction was later shown to be LPS.

The classical generalized Shwartzman reaction (GSR) requires two properly spaced (usually 24 hours apart) intravenous injections of LPS into a rabbit. This results in bilateral renal cortical necrosis, due to fibrin deposition secondary to diffuse intravascular clotting. A precipitous drop in the leukocytes and platelets is essential for the reaction (108). An attempt to define the mechanism of this phenomenon has implicated granulocyte products as the clot-promoting factor (108); other studies implicate stimulation of $\alpha$-adrenergic receptor sites in the microcirculation as the cause of the formation of fibrin thrombi (109, 110). Heparin effectively blocks the GSR (108), but the mechanism is unclear (111).

Since there are striking similarities between the laboratory and pathologic abnormalities of the GSR in rabbits and certain clinical conditions, some investigators have suggested that these are human correlates of the animal model. These conditions include renal cortical necrosis most often associated with a variety of infectious and noninfectious complications of pregnancy (112), thrombotic thrombocytopenic purpura (113), sepsis (114), and post-renal homotransplantation (115). Although these conditions may in fact be human examples of the GSR, the lack of published studies attempting to identify endotoxin in the blood or lesions of such patients leaves the true pathogenesis still in doubt.

## *Effects on Metabolism*

CARBOHYDRATES The injection of LPS into experimental animals produces initial hyperglycemia, which is followed by a prompt decrease in blood sugar to hypoglycemic levels and a concomitant decrease in liver glycogen (116). In the mouse, the hypoglycemia has been shown to be of sufficient magnitude to cause death (117). The mechanism of the hypoglycemia appears to be inhibition of the synthesis of glucose from noncarbohydrate sources (118).

LIPIDS The injection of LPS into rabbits produces hyperlipidemia, which is characterized by an early transitory rise in plasma free fatty acids and, at 24 hours, an elevation of serum cholesterol, serum phospholipids, and plasma triglycerides (119). Patients with gram-negative infections have been shown to have a marked elevation of their plasma triglycerides or free fatty acids (120), although the administration of LPS to volunteers failed to increase lipid levels (J. I. Gallin, R. Levy, S. M. Wolff, unpublished observations). However, the absence of a response was probably due to the small doses of LPS one can safely give to humans.

PROTEINS AND ENZYMES Protein synthesis by the liver is stimulated by LPS injection (121). However, the influence of LPS on specific enzyme activities in the liver indicates a selective effect, as both increases and decreases have been demonstrated (122, 123). LPS injection given to experimental animals causes an increase in several serum enzymes (lactic dehydrogenase, isocitric dehydrogenase, transaminases, and creatine phosphokinase) secondary to tissue damage (124). An interesting observation, of as yet unknown significance for clinical disease, is the fact that rats given repeated injections of endotoxin develop amyloidosis (125).

MINERALS Hypoferremia proportional to the dose of LPS has been demonstrated in man and rats, with the maximum decrease occurring eight to ten hours after injection (126, 127). The total iron-binding capacity of the serum also decreases following LPS administration (128). Endogenous pyrogen liberated by LPS may partially mediate the hypoferremia, since endogenous pyrogen from rabbit polymorphonuclear leukocytes lowers the serum iron concentration of endotoxin-tolerant rats (129). In addition, serum zinc concentrations decrease significantly in a dose-dependent response after LPS injection in the rat (130).

### Nonspecific Resistance to Infection

The administration of LPS can alter resistance to infection by bacteria (131–133), fungi (134–136), viruses (137, 138), and parasites (139, 140) in several animal species. This change in susceptibility to infection is characterized by a transient increased susceptibility (so-called negative phase) followed by an increase in nonspecific resistance to infection. The mechanism of the increased resistance to infection secondary to LPS injection is unclear; however, enhanced macrophage and reticuloendothelial system activity (141), granulocytosis (142), and changes in iron metabolism (136) may be factors. It is clear that the mechanism is complex and that both serum and cellular factors are probably operative (143).

### Effects on the Reticuloendothelial System

The RES was first implicated in the host response to LPS by Beeson in 1947 (36). While a variety of studies have supported the concept that the RES has a role to play, they also have indicated that the relationship between host RES activity and LPS toxicity is complex (144). In general, LPSs are potent stimulators of RES activity, as evidenced by enhanced blood clearance of colloidal carbon (145). Such alterations in the activity of the RES have in turn been related to many of the biological responses of LPS, such as the enhancement of nonspecific resistance (146), the adjuvant effects of LPS (146), the development of endotoxin tolerance (36), and biological detoxification of these substances. The importance of the RES in LPS metabolism is made evident by the fact that the major portion of an injected dose of LPS can be traced to the Kupffer cells of the liver and to the spleen (147).

### Pathology

The administration of single injections of large quantities of LPS causes vesiculation of hepatocytes with progressive necrosis in the centrilobular zone and congestion of the central veins and sinusoids (148). There is a progressive increase in the number, size, and variety of lysosomes, depletion of glycogen, swelling of the Kupffer cells, and fibrin deposition in the sinusoids (149). In the lung there is hemorrhage into the alveolar wall with clumping of polymorphonuclear leukocytes in the pulmonary capillaries (149). Examination of the myocardium by electron microscopy shows marked distortion of the intercalated discs (149).

## CONCLUSION

Great advances have occurred in the purification and definition of the chemical structure of bacterial endotoxins during the past 20 years. Recently, a sensitive in vitro assay for the detection of LPS has been developed. The task now is to sort out the myriad effects of LPS on the intact animal and to define the interaction between

LPS and host tissues at the molecular level. With a greater understanding of the biological properties of LPS, an attempt to devise more effective modes of therapy for some of the clinical effects of such agents in man can possibly be realized.

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# HEMOCHROMATOSIS: COURSE AND TREATMENT

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## INTRODUCTION

The iron storage diseases include all conditions with excess iron in the liver. Two types are sometimes distinguished: hemochromatosis with associated tissue damage and usually cirrhosis, and hemosiderosis where iron overload is seen without liver injury (1). The term hemochromatosis is usually associated with the inborn, idiopathic hereditary hemochromatosis, but other forms of hemochromatosis must also be considered, including that associated with alcoholic liver disease, with blood disorders and with some forms of porphyria.

## IDIOPATHIC HEREDITARY HEMOCHROMATOSIS

This is an inborn error of metabolism associated with a slow accumulation of iron in the body over many years (2, 3). The tissues contain enormous quantities of iron, of the order of 20–60 g, compared with a normal total body content of about 4 g. These large amounts can only have accumulated as a result of increased intestinal absorption over a long period. If 10 mg dietary iron were retained by the tissues daily, it would take about 14 years for 50 g to accumulate.

### *Presentation*

The disease is essentially one of males, being ten times as frequent as in females (3, 4). The peak incidence is between ages 40 and 60. It is usual to see slaty pigmentation maximal in the axillae, groins, and old scars (5). The liver is enlarged and firm, but signs of portal hypertension and liver failure are rare at presentation. Clinical diabetes is present in two thirds of the cases (6). This may be complicated by nephropathy, neuropathy, peripheral vascular disease, and retinopathy. Pituitary function is impaired to a variable extent in about two thirds of patients. Failure of at least one trophic hormone can be related to iron deposition in the anterior pituitary and not to severity of liver disease, or to degree of abnormality of iron or estrogen metabolism (7).

Cardiac symptoms are frequent in younger patients and consist of arrhythmias or right-sided heart failure.

The diagnosis is confirmed by demonstrating a high serum iron value, about 200 μg/dl, with 90% saturation of serum iron-binding protein. Serum ferritin is increased.

Increased iron can be demonstrated in the tissues. Needle biopsy of the liver is the most satisfactory method when a pigmentary cirrhosis is suspected (8). The amount of iron in the needle biopsy specimen may be quantitated using a simple chemical method (9). Results correlate with total body storage of iron as measured by chelation methods. If the liver iron is less than 1.5% dry weight in a cirrhotic liver, the condition is unlikely to be idiopathic hemochromatosis. Liver biopsies include those from skin and stomach. Bone marrow shows increased deposits of stainable iron.

Chelation methods using desferrioxamine or diethylene triamine pentaacetic acid may be used to demonstrate increased iron stores, but, owing to their complexity, they have been replaced by such simple methods as the estimation of serum ferritin.

### *Treatment*

Iron can be removed from the tissues by venesection and can be mobilized from stores at rates as high as 130 mg per day (10, 11). Radioactive iron studies showed that the already-raised serum iron turnover in hemochromatosis is increased further by venesection. Blood regeneration is extraordinarily rapid, and hemoglobin production increases to six to seven times its normal rate (10). Large quantities of blood must be withdrawn; a phlebotomy of 500 ml removes only 250 mg of iron, whereas the tissues contain up to 200 times this amount. Depending on the initial iron stores, the amount necessary to effectively reduce them varies from 7 to 45 g (11). Venesection of 500 ml should be carried out weekly or, if possible, twice weekly in cooperative patients. The approach to treatment should not be too leisurely (12). Phlebotomies are continued for about one to two years until the hemoglobin and serum iron levels fall. Liver biopsy is a good check that de-ironing is complete. Siblings with significant iron deposition should also be treated by venesection until the liver biopsy shows absence of stainable iron (13).

The most effective iron chelating agent is ferrioxamine. This only removes about 10 to 20 mg iron daily and the amount is reduced when administration is repeated. These results are not comparable to the effect of phlebotomy, and the former means of treatment is unnecessary in patients with idiopathic hemochromatosis.

A low iron diet is impossible to achieve. An alternative is to prescribe large doses of phosphate which prevent iron absorption by forming a precipitate and so increase fecal excretion of dietary iron.

Alcoholism seems to increase the toxic effect of iron on the liver in patients with idiopathic hemochromatosis, and these patients should be encouraged to totally abstain from alcohol.

## *The Course of the Disease and the Effects of Venesection Therapy*

PROGNOSIS The prognosis and course of the undiagnosed case cannot be stated nowadays, for once the disease is recognized, treatment is instituted immediately with venesection. The prognosis has therefore inevitably become better over the last twenty years. In one series, 40 patients receiving venesection therapy were compared with 18 untreated patients. Although the analysis was retrospective, patients receiving phlebotomy presented between 1950 and 1965 and the untreated between 1939 and 1960. The mean survival for the phlebotomy group was 8.2 years, while the untreated group had a mean survival of 4.9 years (14). In a similar retrospective analysis of 26 nonalcoholic patients with hemochromatosis, a significant difference in mortality was found: 3 of the 19 patients in the treated group died, compared to 4 of 7 in the untreated group (15). The treated patients had a greater sense of well-being, skin pigmentation decreased, and the patients gained weight.

### *Laboratory Tests*

Hemoglobin concentration during treatment usually fluctuates between 11 and 13 g/100 ml. Slightly lower values are occasionally seen, but transiently, and the red cells do not become hypochromic. Serum iron fluctuates slightly below the pretreatment level. The end of venesection treatment is heralded by serum iron falling rapidly from the normal to the subnormal range, with the total iron binding capacity simultaneously rising (12). A progressive fall in hemoglobin concentration soon follows. Several more venesections are usually carried out over the next two months, removing a further 1 to 2 g of iron.

### *Iron Store Depletion*

Complete iron store depletion is ultimately indicated by hypochromic anemia, hypoferremia, and reticulocytopenia persisting for several weeks after the last venesection. To prevent iron reaccumulation, treated patients should have a venesection every three to four months throughout life.

Serum ferritin levels have recently been introduced as a measure of body iron stores and of the effect of treatment in iron storage states. Ferritin is an iron storage protein found mainly in liver, spleen, and bone marrow, and can be demonstrated by an immunoradiometric method in serum (16). The level is generally proportional to body iron stores (17). It correlates inversely with iron absorption (18). High values in hemochromatosis correlate with the liver iron concentration (19). However, if there is hepatocellular injury, this is indicated by the ratio between serum ferritin and the transaminase concentration (19). Serial measurements of the ratio are of value in following the course of iron overload (19). Serum ferritin may be an ideal measurement for monitoring the progress of patients with idiopathic hemochromatosis during treatment. It may also prove invaluable for the detection of affected relatives of patients with this disease, being more convenient than the chelation test currently employed, although this requires further investigation (19). Ferritin: transaminase ratios of more than 60 generally indicate marked iron excess, while those of less than 20 are associated with normal iron stores and those of less than 10 with suboptimal stores.

### *Hepatocellular Function and Fibrosis*

The hepatic lesion of hemochromatosis culminates in a macronodular cirrhosis. The histological features are characteristic: fibrous septa surround groups of lobules and irregularly shaped nodules, and the fibrosis is often described as "holly leaf" in appearance (8). Progression to clinically overt portal hypertension with bleeding esophageal varices is unusual.

During the course of venesection treatment, the iron disappears from the liver cells, and this is confirmed by needle biopsies of the liver which show histological disappearance of iron, and also quantitatively by a reduction in the liver iron concentration (9). Occasionally, with intensive venesection therapy, the "cirrhosis" of idiopathic hemochromatosis has been shown by serial liver biopsies to reverse completely (20). This is unexpected, as cirrhosis is generally regarded morphologically as an irreversible condition. It seems likely that the liver lesion of hemochromatosis is not, in fact, a true cirrhosis, for intact nodules are inconspicuous and, even when present, the architecture of most lobules seems to be preserved.

Hepatocellular failure is unusual and usually terminal in untreated patients. After venesection therapy, liver size decreases and liver function tests return to normal (14).

### *Primary Hepatocellular Carcinoma*

Primary liver cancer is about twice as common in hemochromatosis as in other forms of cirrhosis in the same community (21). It is apparently increasing, as other complications of hemochromatosis, such as diabetes, come under control. Effective de-ironing does not seem to prevent its development (22, 23). It should be suspected if the patient shows unexpected clinical deterioration with rapid liver enlargement, abdominal pain, and ascites. Hepatic scanning should be performed on all patients with hemochromatosis at the time of diagnosis and repeated if primary liver cancer is suspected. Serum $\alpha$-fetoprotein levels should also be performed, but there seem to be no reports so far of values in liver cancer complicating hepatic iron overload.

### *Diabetes*

Clinical diabetes is present in about two thirds of cases of hemochromatosis (6). It may be easy to control or resistant to huge doses of insulin (6). This is due both to the insulin sensitivity of cirrhosis and to pancreatic damage from iron (24). The diabetes of hemochromatosis may be in part genetically determined, the additional factor being siderotic damage of the pancreas, making manifest the underlying susceptibility to diabetes (25). Venesection treatment usually has little effect on the insulin sensitivity, although occasionally a dramatic improvement is seen (6). In one large series, only 40% of patients with diabetes showed improvement of carbohydrate tolerance following venesection therapy (6).

### *Other Endocrine Changes*

Testicular atrophy is a frequent feature, being found in 13 of 23 patients with idiopathic hemochromatosis in one series (7). This may be related to loss of pituitary gonadotrophins rather than to estrogen-androgen imbalance, as in cirrhotic patients. The testicular failure is little affected by venesection therapy and testicular substitution may be needed.

### *Cardiac Failure*

In 1955, in a review of 700 cases of hemochromatosis, cardiac failure was the third commonest cause of death (3). However, with the advent of venesection therapy, this appears to be less common (14). Once heart failure has developed, it shows a poor response to conventional methods of cardiac therapy.

### *Arthropathy*

The specific arthropathy of hemochromatosis is associated with calcium pyrophosphate crystals in the synovia (chondrocalcinosis; "pseudogout") (26). The distribution is the same as in rheumatoid arthritis, but the deformities of rheumatoid arthritis are not seen. Clinical or radiological improvement does not follow venesection treatment (26, 27).

## CIRRHOSIS OF THE ALCOHOLIC

Increased liver iron is seen in cirrhosis of the alcoholic. In these patients, iron deposition is rarely as great as in the idiopathic disease. Hepatic histology shows the features of alcoholism (particularly alcoholic hepatitis) as well as iron. Diagnostic difficulties arise when a patient carrying the gene for idiopathic hemochromatosis is also a heavy drinker.

The prognosis of cirrhosis of the alcoholic with iron deposition is considerably worse than for patients with idiopathic hemochromatosis. Iron deficiency soon follows venesection. It is uncertain how much the iron overload aggravates the underlying liver disease. In two series, venesection treatment has not been found helpful (15, 28). In a prospective study of patients with alcoholic cirrhosis whose liver biopsies showed greater than 10% of the liver cells to contain iron, depletion of hepatic iron stores by phlebotomy was not shown to influence post-randomization morbidity or survival (29). Most of these patients had iron stores under 10 g, as determined by phlebotomy.

## AFTER PORTACAVAL SHUNT

After portacaval anastomosis there is rapid deposition of large amounts of hemosiderin in patients who previously were free of iron deposits in the liver (30). All the features of idiopathic hemochromatosis may be noted, including skin pigmentation and diabetes. This association has been confirmed in a prospective controlled trial with analysis of hepatic biopsy specimens before shunting and four years later (30). Other workers, however, are not so sure of the association (31, 32), though iron absorption is undoubtedly increased after portacaval shunt (32). In any case, the iron deposits are modest and treatment by venesection is rarely indicated.

## CHRONIC ERYTHROPOIETIC DISORDERS

Increased erythropoietic activity is a dominant stimulus for increasing alimentary iron absorption, and this continues even in the presence of anemia and large iron stores. Hemochromatosis is therefore associated with many chronic hemolytic states, particularly thalassemia and sickle-cell anemia, and even congenital spherocytosis (23). It is also seen in the rare, often hereditary, hypochromic anemia with siderosis (33). The siderosis is enhanced by misdirected iron therapy, whether oral or parenteral, and particularly by multiple transfusions.

It is recognized clinically by increasing skin pigmentation and hepatomegaly. Liver failure and frank portal hypertension are rare complications. The fasting blood glucose level is raised, but clinical diabetes is excessively rare. The complication has particularly increased in thalassemia since blood transfusions are more readily available and survival has been prolonged into adolescence and early adult life. Hemochromatosis is now a major cause of death in children with thalassemia.

Patients with this type of hemochromatosis obviously cannot be treated by venesection. Chelating agents, such as desferrioxamine or *diethylene triamine pentaacetate,* cause urinary losses of the order of 10 to 50 mg daily. Nevertheless, in a prospective controlled trial conducted in children with homozygous thalassemia, a significant difference was seen in liver iron and fibrosis in the treated group (34).

## PORPHYRIA CUTANEA TARDA

This chronic acquired disorder of porphyrin metabolism is characterized by skin photosensitivity, pigmentation, and hirsutism. Biochemically, it is characterized by excessive hepatic synthesis and fecal and urinary excretion of uroporphyrin.

Iron stores are markedly increased (35, 36). There may also be liver damage, but this is related more to excess alcohol intake than to the modest iron overload. Nevertheless, venesection therapy does seem to be beneficial.

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# TUMOR ANTIGENS AND TUMOR-HOST RELATIONSHIPS

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## INTRODUCTION

The view that immunological reactions play a role in modifying or even controlling malignant disease has now gained wide acceptance. This has developed from well-substantiated investigations showing that many, although by no means all, experimental animal tumors induced by chemical carcinogens or oncogenic viruses, as well as those of unknown (spontaneous) etiology, elicit immune rejection responses against the tumor (1, 2). Consequently, hosts preimmunized against these tumors (e.g. by implantation of radiation-attenuated cells) will reject a subsequent challenge with the same tumor.

Comparable evidence for host defenses in human malignant disease is not available, apart from limited studies indicating that autografted tumor cells do not readily "take" (3). There are, however, a small number of impressive examples from clinical investigations suggesting, but not proving, that in certain circumstances immunological reactions may control malignant disease. These include reports of spontaneous remissions in melanoma, neuroblastoma, and renal cell carcinoma (4), as well as remissions occurring in Burkitt's lymphoma patients receiving minimal chemotherapy (5). Conversely, there is substantial evidence of increased incidences of malignant tumors in patients receiving continuous immunosuppressive therapy following organ transplantation (6). Finally there is an increasing number of studies showing that procedures designed to heighten patients' general immunocompetence or to specifically boost tumor-immune responses may have therapeutic potential.

Broadly speaking, however, the major body of evidence supporting the concept that human tumors express neoantigens similar to those detected in experimental animal tumors has been derived from in vitro assays of cell-mediated and humoral antibody responses to tumor cells (7). In addition there are a number of reports showing that cancer patients exhibit delayed hypersensitivity responses to tumor extracts (8).

Cell-mediated immunity is generally considered to be primarily responsible for tumor rejection responses elicited against experimental animal tumors such that, for example, it is possible to transfer resistance against transplanted tumors to normal compatible hosts with lymphoid cells from tumor-immune and, in some instances, tumor-bearing donors (9). It is possible to monitor these tumor-specific immune responses in vitro by employing a variety of procedures for evaluating the reactivity of sensitized lymphoid cells. One assay widely used for this purpose is the microcytotoxicity test in which the cytotoxic or cytostatic effects of sensitized lymphoid cells are determined by their capacity to inhibit growth of tumor cells in culture (10). This test has been widely held to detect relevant tumor-immune reactions since in many studies on experimental animal tumors it was possible to demonstrate cell-mediated responses with specificities identical to those evaluated in vivo by rejection of tumor transplanted into syngeneic hosts (1, 7). The microcytotoxicity assay, which involves visual counting of surviving target cells or variants employing radioisotope-labeled tumor cells, has also been used to evaluate the cytotoxicity of peripheral blood leucocytes from cancer patients. From these studies evidence has accumulated showing that a wide range of tumor types exhibit neoantigens against which patients direct cell-mediated immunity (7). The specificities of the tumor antigens detected in these tests differ, however, from those identified in many experimental animal tumors in that they are related to the histological tumor type. For example, blood leucocytes from a patient with colon carcinoma were cytotoxic for the patient's own tumor and other colon carcinoma cells, but not for histologically different tumors (7, 10).

Two points need to be considered in evaluating the significance of the many studies in which cell-mediated immune responses to human tumor-associated antigens have been defined by in vitro leucocytotoxicity testing. First, there are now a number of reports in which the validity of the assay has been questioned since leucocytes from normal donors and non–cancer patient controls may be reactive in these tests (11, 12). Second, it is clear that in vitro cytotoxicity by sensitized lymphoid cells for plated tumor cells is not a necessary correlate of a tumor rejection response. This is illustrated by studies with chemically induced and spontaneously arising rat mammary carcinomas in which lymphoid cells from tumor-bearing and tumor-free donors were found to be sensitized towards tumor-associated antigens since they were cytotoxic in vitro for tumor cells (13). Nevertheless, these tumors were only weakly or nonimmunogenic since this property was not detected when assessed by their capacity to induce immunity in normal syngeneic rats toward transplanted tumor cells.

Despite these reservations, evidence for the existence of neoantigens in human tumors is considerable and is based upon data evaluated with a variety of in vitro tests. In addition to leucocytotoxicity, cell-mediated reactions have been defined by inhibition of leucocyte migration (14, 15) or leucocyte adherence (16), and by lymphocyte stimulation with tumor cells or extracts (17). Alternatively, skin tests for delayed hypersensitivity reactions to tumor extracts have been used to study the cellular immune response of patients to tumor-associated antigens (8).

## IMMUNE RESPONSES IN THE TUMOR-BEARING HOST

Experimental animal studies have established that hosts with a tumor developing at one site, which is beyond host control, can nevertheless reject a concomitant challenge with the same tumor implanted at another site, providing that this challenge is not too great (18–21). This indicates that the tumor-bearing host has developed an effective immune response to the second tumor challenge and implies that the response to the primary tumor was too weak or developed too slowly to cause its rejection. Alternatively, host factors may have interfered with the effective mediation of tumor immunity. The first explanation has been formulated as "sneaking through," in which it is envisaged that a nascent tumor may not initially stimulate the immune system and by the time sufficient tumor is present to provide an effective stimulus to the host's immunological apparatus the established tumor may proliferate at a rate such that its growth outstrips immunological control (22). The second hypothesis proposes that the nascent tumor may provoke a specific immune response through host recognition of tumor-associated antigens, but this may be diminished through the intervention of suppressor factors, thus providing the tumor with an escape mechanism from immunological control. This concept was originally formulated in terms of circulating humoral factors by the Hellströms, who established that lymphoid cells from tumor-bearing as well as tumor-free donors were cytotoxic in vitro for the appropriate target cells, but that tumor-bearer serum specifically abrogated these effects (7, 23). Since the original description of serum abrogation of tumor-directed cell-mediated immunity with Moloney virus-induced murine sarcomas (24), this phenomenon has been demonstrated with a wide range of experimental animal and human tumors. In addition, the effects of tumor-bearer serum have been demonstrated using other in vitro procedures to monitor cell-mediated immunity to tumor cells including inhibition of leucocyte adherence (16) and lymphocyte stimulation (17).

The roles of specific as well as nonspecific factors in producing defects of cell-mediated immunity in the tumor-bearing host are still poorly understood, and in addition to circulating serum factors, suppressor lymphoid cells and products released from these cells may be involved (25, 26). It should also be recognized that the factors, or combination of factors, which abrogate immunity to a nascent tumor may be totally different from those involved in the so-called eclipse phenomenon in which tumor-immune responses are suppressed when tumors develop beyond a certain size. This latter event has been demonstrated, for example, by showing that the capacity of mice bearing a transplanted tumor to reject a contralateral challenge with the same tumor declines as the primary implant increases in size, resistance being restored following removal of the tumor (27–29). Defects in tumor-directed cell-mediated immunity, as distinct from a general immunological anergy, in animals with progressively growing tumors have also been detected by reduction in the capacity of tumor-bearer lymphoid cells to adoptively transfer resistance to normal compatible hosts (29, 30), and by monitoring delayed hypersensitivity responses to tumor cells (31). There is also considerable evidence from experimental animal studies to show that the in vitro cytotoxicity of tumor-bearer lymphoid cells diminishes with progressive tumor growth (29, 32), including one lesion involving T-cell responses (29).

## NATURE OF SERUM FACTORS ABROGATING TUMOR IMMUNITY

Interference with cell-mediated immunity in the tumor-bearing host can be envisaged (Figure 1) as resulting from serum factors interacting with and masking neoantigens on tumor cells, thus preventing their recognition by sensitized lymphoid cells (blocking reactions). Alternatively, these factors may directly interact with effector cells and inhibit their reactivity (inhibitory reactions). In the tumor-bearing host it is likely that the involvement of different mechanisms will vary according to the extent of the disease. Moreover, effects produced at the level of the target tumor cell, e.g. through interaction with a tumor-specific antibody, may lead to the release of factors which influence the reactivity of sensitized lymphoid cells (Figure 1).

### *Blocking Reactions at the Tumor Cell Surface*

The original proposal that serum abrogation of lymphocyte cytotoxicity was mediated by antibody binding to neoantigens expressed at the tumor cell surface was based upon the finding that with murine Moloney virus-induced sarcomas, blocking factor could be neutralized by goat anti-mouse 7S immunoglobulin (24). Also,

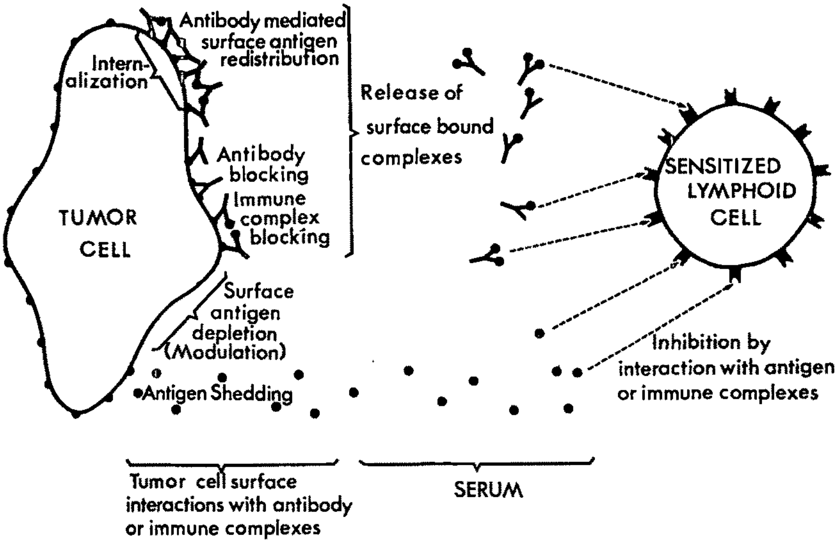
Antibody mediated surface antigen redistribution
Intern-alization
TUMOR CELL
Antibody blocking
Immune complex blocking
Surface antigen depletion (Modulation)
Antigen Shedding
Release of surface bound complexes
SENSITIZED LYMPHOID CELL
Inhibition by interaction with antigen or immune complexes
Tumor cell surface interactions with antibody or immune complexes
SERUM

*Figure 1* Schematic representation of possible interactions of humoral factors interfering with cell-mediated immune reactions.

blocking factor in the serum of tumor-bearing animals has been isolated by Sephadex G-200 and sucrose density gradient centrifugation in fractions containing 7S immunoglobulins (33). This interpretation was viewed as inadequate, however, since serum blocking activity was found to decline rapidly in animals whose tumors spontaneously regressed (23, 24) or where a developing tumor was surgically removed (34). A similar correlation was obtained in human studies (35) in which blocking activity was present in 85% of patients with clinical evidence of tumor, but in only 14% of patients in whom there was no clinical evidence of tumor. These observations led to the suggestion that serum factors blocking tumor cell surface antigens may be tumor-specific immune complexes, and evidence to support this has been obtained in three ways:

(*a*) Dissociation of serum blocking factor into low and high molecular weight components with subsequent loss of activity which was restored on recombination.
(*b*) In vitro formation of immune complexes with enhanced blocking activity.
(*c*) Neutralization of tumor-bearer serum blocking activity by the addition of excess tumor-specific antibody.

The first approach was originally employed to show that the blocking factor in the serum of mice bearing Moloney virus-induced sarcomas could be absorbed onto intact tumor cells, eluted with low pH buffers, and then separated by a membrane ultrafiltration procedure into high molecular weight (greater than 100,000) and low molecular weight (10,000 to 100,000) fractions (36). Neither of these separated fractions alone displayed blocking activity when added to cultured Moloney sarcoma cells, although activity was restored upon recombination. This procedure was also adopted to characterize blocking factors eluted directly from surgical specimens of human tumors. It was shown that low pH buffer eluates were blocking, but this activity was lost when the eluates were separated into "low" and "high" molecular weight fractions (37). Blocking was restored on recombination of the two fractions, and in addition, a similar effect was obtained when tumor cells were first exposed to the high molecular weight fraction (putative antibody) and then low molecular weight fraction (putative antigen), but not vice versa. Tumor-specific antigen and antibody were not unequivocally identified in the fractions isolated in the low pH buffer eluates of tumor tissue and this point deserves further study. It should be appreciated, however, that tumor-bound immunoglobulins have been isolated by several procedures including extraction of tissues with low pH buffers or hypertonic salt solution (38, 39), and in tests on malignant melanoma it was reported that specific antibody was present in the eluates (39). Also, elution of sarcoma cells with pH 3 buffers increased their capacity to induce stimulation of peripheral blood lymphocytes, the view being that antibody or immune complexes were being removed, thus rendering the tumor cells more responsive (17).

Further support for the view that tumor-bearer serum blocking reactions are mediated by specific immune complexes comes from tests showing that complexes formed by the addition of soluble tumor-specific antigen (papain-solubilized membrane), prepared from a transplanted rat hepatoma, to serum taken from rats following surgical removal of tumor grafts, were effective in blocking tumor cells from cytotoxic attack by immune lymph node cells (40). Also, tests on neuroblastoma patients in which blocking factors containing immune complexes, together with either free tumor antigen or antibody, were prepared by adding tumor antigen (supernatant from tumor cell cultures) to serial dilutions of serum from patients in remission, indicated that abrogation of the cytotoxicity of peripheral blood lymphocytes was most efficient at antigen-antibody equivalence (41).

Finally, a third approach employed for defining the role of immune complexes in tumor-bearer serum blocking effects has been to demonstrate that this activity can be abolished in the presence of excess tumor antibody. Antibody-mediated neutralization (unblocking) of the blocking activity of tumor-bearer serum was initially reported with murine Moloney virus-induced sarcomas, where blocking effects were counteracted by mixing tumor-bearer serum with serum taken from mice whose tumors had spontaneously regressed (42). Unblocking effects have been similarly described with other experimental tumor systems employing a variety of tumor-specific antisera, including sera taken from hosts surgically cured of a developing tumor graft (43–45) as well as antisera produced by immunization of syngeneic or xenogeneic hosts with tumor cells (46). Similarly, serum from clinically symptom-free patients is "unblocking, " since addition of these sera to tumor-bearer serum reduced their capacity to block tumor cells from cytotoxic attack by peripheral blood lymphocytes (47).

Blocking studies indicate that tumor cell killing by sensitized lymphoid cells can be abrogated by exposure of tumor cells to tumor-specific antibody, or more effectively, to tumor-specific immune complexes. This effect may be produced through masking of cell surface-expressed neoantigens on tumor cells which prevents their recognition by sensitized effector cells (Figure 1). Alternatively, antibody or immune complex binding to tumor cells may result in depletion of tumor antigen at the cell surface. This may occur through a process of modulation whereby antibody binding to tumor antigen at the cell surface alters their surface distribution, inducing polarization and internalization of these receptors (48). Also, binding of antibody-containing moieties to tumor antigen may induce or accelerate their shedding from the tumor cell surface. This might lead to a depletion of cell surface receptors available for interaction with sensitized lymphoid cells, and at the same time antigen-containing material shed from the tumor cell may specifically inhibit the reactivity of effector cells (Figure 1).

### *Inhibition of Lymphoid Cell Reactivity*

An alternative pathway by which serum factors in the tumor-bearing host may abrogate cell-mediated immunity involves specific desensitization (inhibition) of cytotoxic lymphoid cells through interaction with tumor antigen, either in free form or as immune complexes (Figure 1). This type of inhibitory response has been demonstrated with a number of experimental animal tumors, the in vitro cytotoxicity of sensitized lymph node or spleen cells being abolished by pre-exposure to tumor-bearer serum (49–51). Serum from patients with colon carcinoma also specifically inhibited the in vitro cytotoxicity of peripheral blood lymphocytes, this being viewed as an effect on T-lymphocyte responses since the reactive cell population formed spontaneous rosettes with sheep erythrocytes (52). These findings are complementary to studies on patients with disseminated malignant melanoma in which peripheral blood lymphocytes initially exhibited little cytotoxicity for melanoma cells in vitro, but reactivity was detected after repeated washing. Furthermore, the cytotoxicity of washed peripheral blood lymphocytes could be inhibited again by addition of the patient's serum (53).

Circulating tumor antigen and/or immune complexes have been implicated in this type of inhibitory response leading to a reduction in the cytotoxicity of sensitized lymphoid cells, since serum activity rapidly decreased in animals rendered tumor-free, e.g. by surgical resection of tumor grafts, and was not detected in tumor-immune serum (46, 49). Furthermore, the lymphoid cell inhibitory activity of serum from rats bearing transplanted hepatomas could be neutralized by the addition of tumor-specific antibody (46). Additional support for a role for tumor antigen in this type of inhibitory response is provided by tests in which the cytotoxicity of sensitized lymph node and spleen cells was abrogated by pre-exposure to tumor antigen preparations. For example, the cytotoxicity of lymph node cells from rats immunized against transplanted rat hepatomas could be specifically abrogated by pre-exposure to papain-solubilized tumor antigen fractions (49). These tumors also express cross-reactive fetal antigens at the cell surface (54) and the tumor-bearing host responds to these antigens, producing sensitized lymphoid cells which are cytotoxic for a range of tumor target cells (32, 55). These effector cells, as well as lymph node cells from multiparous rats (which are sensitized to fetal antigens), could also be inhibited by exposure to soluble fetal antigen-containing extracts prepared from both tumor and fetal tissues (55, 56). Similar approaches have been employed to show that the cytotoxicity of lymphoid cells from animals bearing Moloney virus-induced sarcomas (51, 57), as well as virus-induced mammary carcinomas (50, 58), can be abrogated by exposure to tumor antigen employing either tumor cell membrane extracts or even crude as well as purified tumor virus preparations. Akin to these experimental tumor studies, extracts of human tumors, in particular carcinoma of the colon and malignant melanoma, prepared by papain solubilization of tumor membrane, specifically inhibit the in vitro cytotoxicity of peripheral blood leucocytes (59, 60).

## IN VIVO ROLE OF SERUM FACTORS

### *Monitoring Serum Factors During Tumor Growth*

In vitro studies on the influence of tumor-bearer serum on lymphocytotoxicity, and other allied techniques for measuring cell-mediated immunity such as leucocyte migration and leucocyte adherence tests (14, 16), suggest that circulating tumor antigen and/or immune complexes may play a biologically important role in the tumor-host relationship through interference with cellular immunity. The crucial question still unresolved is whether the effects of tumor-bearer serum observed in vitro do play an active role in vivo. In considering this possibility, it should be appreciated that the serum levels of tumor antigens and specific immune complexes show marked variations when different experimental tumor types are compared, and they also vary according to the stage of tumor development. This is illustrated by tests on a transplanted rat hepatoma in which free circulating tumor antigen was present in serum taken during the initial phase of tumor growth (61). As a consequence of host immune responses to neoantigens expressed upon this tumor, the level of free tumor antigen in serum then rapidly decreased and at this stage immune complexes were identified. Finally, the terminal phase of tumor growth was associated with high serum levels of tumor-specific immune complexes in antibody excess (61). Allied with these findings, serum taken during the early phase of tumor growth inhibited the cytotoxicity of immune lymph node cells, but exhibited little blocking activity at the target tumor cell level. Although serum taken during later phases of tumor growth did exhibit blocking activity, the critical event which may contribute to immunological control of this tumor appears to be mediated by serum-borne tumor antigen. The pattern of release into serum of tumor-associated factors during growth of other experimental animal tumors has differed to some extent, although an early and rapid appearance of circulating tumor antigen has been observed with other tumors. With a transplanted rat sarcoma, for example, serum levels of tumor antigen increased with progressive tumor growth, but then rapidly declined following surgical resection of the tumor (62, 63). Also, when growth of this sarcoma was suppressed by a contralateral injection of tumor cells admixed with BCG, treated animals developed a tumor-specific antibody response and serum-borne tumor antigen was not detected (64). This can be compared with tests on a murine sarcoma showing that concomitant immunity, defined by the tumor-bearing host's capacity to reject a contralateral challenge with the same tumor, diminished as the primary tumor implant developed. Tumor immunity was rapidly restored, however, following surgical removal of the primary tumor graft (28).

Circulating tumor antigen has been identified in human cancer as exemplified by the presence in serum of carcinoembryonic antigen (CEA) in patients with various types of malignancy, but especially carcinoma of the colon (65), and $\alpha$-fetoprotein ($\alpha$FP) in hepatocellular carcinoma (66, 67). Although these tumor-associated fetal products are not thought to play a significant role in the immunobiology of the tumor-bearing patient, they draw attention to the likelihood that other tumor antigens will be detected in the serum of cancer patients. Progress is limited, however, because there are as yet few reliable methods for detecting serum-borne human tumor antigens other than for products such as CEA and $\alpha$FP. In this context, serum fractions from patients with carcinoma of the breast and bladder modify the migration of patients' leucocytes (68), the reactions being comparable to those produced with tumor cells or tumor extracts (14), and imply that these sera contain tumor antigen. Also, in the bladder carcinoma series, serum reactivity could be related to the stage of the disease (68). Circulating tumor-associated products in immune complexes have also been identified in the serum of patients with neuroblastoma (41) and Burkitt's lymphoma (69). The techniques employed in these studies require further development to improve their specificity and reproducibility, but this approach suggests that it should be possible to develop sensitive assays for detecting tumor antigen which might have considerable potential for sequential monitoring of patients receiving therapy or for detecting early and/or residual disease.

### *Abrogation of Tumor Immunity*

The concept that tumor antigen released into the circulation of the tumor-bearing host may play a role in abrogating tumor immunity has gained support from experimental studies showing that this type of effect can be produced in vivo. For example, concomitant immunity in mice bearing syngeneic implants of a transplanted sarcoma, or the immune response developing following surgical resection of tumor grafts, was depressed following injection of radiation-killed tumor cells (19, 27, 28). Also, intraperitoneal injection of tumor antigen, either in the form of radiation-killed tumor cells or using 3 M KCl-solubilized tumor extracts, depressed the immunotherapeutic response mediated by contralateral injection of tumor cells admixed with BCG in rats carrying a transplanted sarcoma (P. J. McLaughlin, unpublished findings). Similar tests using tumor-bearer serum to abrogate tumor immunity have not proved so conclusive, since both suppression and enhancement of tumor growth have been observed (70, 71). This is probably due to qualitative or quantitative differences in the levels of tumor antigen, antibody, or immune complexes in the test sera employed, and in almost all cases these factors have not been defined. This can be illustrated by tests on the abrogation of immunity to a transplanted murine sarcoma in which the most consistent response was achieved employing tumor-bearer serum taken from donors immunosuppressed by whole-body irradiation in order to achieve high levels of circulating tumor antigen (72).

### *Neutralization of Serum Factors Abrogating Tumor Immunity*

An alternative approach to resolving whether or not tumor antigen-containing factors are relevant in the tumor-host relationship has been to evaluate to what extent tumor growth in vivo can be influenced by procedures designed to counteract blocking/inhibitory substances in serum or other tissue fluids, e. g. lymph. This type of investigation has not yet been adequately developed, but, for example, regression of both primary and transplanted tumors induced by polyoma virus has occurred following repeated administration of tumor antiserum (44). Even though tumor regression was accompanied by a loss of serum blocking activity, it cannot be concluded that tumors regressed because of immune serum neutralization of serum factors, since passively transferred antibody may itself mediate several responses, including complement and cell-dependent antibody killing. These studies do indicate, however, that monitoring serum factors interfering with tumor-directed cell-mediated immunity may be of considerable value in evaluating the effectiveness of immunotherapy or, indeed, responses to conventional therapy. This has already been referred to in tests on the immunotherapy of a rat sarcoma by contralateral injection of tumor cells admixed with BCG (64). When tumors regressed following treatment, a tumor-specific antibody response developed, and no serum-borne antigen was detected. Comparably, in a rat sarcoma system, when immunotherapy by contralateral injection of tumor cells admixed with BCG was successful, rats did not develop blocking activity (M. J. Embleton, in preparation). In this context, an assay of circulating serum factors which abrogate the cytotoxicity of peripheral blood leucocytes from patients with disseminated malignant melanoma has been employed in a pilot study to monitor the effectiveness of immunotherapy in combination with chemotherapy (73). This is in accord with the conclusions from sequential studies on patients with malignant melanoma, indicating that the presence of serum blocking activity reflects an unfavorable prognosis (7, 74).

## CONCLUSION

Interference with cell-mediated immune responses by humoral factors has been well established by employing in vitro assays to monitor these effects. These studies demonstrate that tumor immunity can be suppressed in either a specific or nonspecific manner by tumor antigen, antibody, or immune complexes, although the contribution of these factors in modifying responses in the tumor-bearing host has not been adequately defined. There is, however, growing evidence to indicate that released tumor antigen may be important in modifying immune responses to nascent tumors. Two developments can be formulated from these observations. First, in designing immunotherapy protocols it may be necessary to define conditions whereby elimination or counteraction of serum blocking and inhibitory activities can be achieved, since this may enhance the therapeutic effect. Second, monitoring of circulating serum factors, especially tumor antigen, may have diagnostic and prognostic value.

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# MITRAL VALVE PROLAPSE[1] ❖7190

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## INTRODUCTION AND HISTORICAL BACKGROUND

Mitral valve prolapse is a condition in which one or both mitral valve leaflets prolapse or balloon back into the left atrium during left ventricular systole, and which may or may not be accompanied by mitral regurgitation. Because of the clinical findings, it has also been called the mid-systolic click–late systolic murmur syndrome, mid-systolic click syndrome, or late systolic murmur syndrome; because of the pathological and surgical findings it has also been called the floppy valve syndrome. Cuffer & Barbillon (1) in 1887 first described the "mid-systolic click" (2) as a "bruit de galop mesosystolique." In 1913 and again in 1932 Gallavardin (3, 4) attributed these clicks to pleuropericardial adhesions, thereby influencing several generations of authors to think they were extracardiac in origin and hence "innocent" (2, 5–11). This thinking prevailed until the early 1960s in spite of the fact that Griffith in 1892 (12), Hall in 1903 (13), and White in 1931 (14) attributed the click and/or late systolic murmur to mitral valve dysfunction. In 1961 Reid (15) again postulated that the click and murmur were of mitral valve origin, the click or "chordal snap" being due to sudden tautening of previously lax chordae, and the

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murmur, when present, to mitral regurgitation. In 1963, Barlow et al (16) demonstrated angiographically the presence of mitral regurgitation in patients with the "click-murmur" syndrome. Subsequently a myriad (17–111) of reports have confirmed and expanded these observations, and have begun to delineate an evolving syndrome that is still poorly understood in many respects. Indeed, in the past five years the literature on this subject has become as voluminous as the prolapsed leaflets themselves (see section on pathology and pathogenesis, and Figure 1). This fact has dictated that the literature review be selective.

## ETIOLOGY AND ASSOCIATED CONDITIONS

Mitral valve prolapse or the "click-murmur" syndrome has been described in Marfan's syndrome (16–22), in Turner's syndrome (17, 20), in hypertrophic cardiomyopathy (19), in patients with a history of acute rheumatic fever (16, 19, 23–26), following mitral commissurotomy (19, 26), in coronary artery disease (21, 25–29), as well as in association with ruptured chordae (30) and secundum atrial septal defect (17, 26, 31–35). However, the majority of cases are idiopathic (26), and in many instances prolapse occurs in familes (17, 21, 23, 26, 35–40), apparently with a Mendelian dominant inheritance (36, 39, 40). Many patients with the idiopathic or familial form of prolapse have thoracic or other skeletal abnormalities reminiscent of Marfan's syndrome (42–44). As a result, some authors have suggested that the idiopathic or familial form of the condition is a forme fruste of Marfan's syndrome (44, 45). This review focuses on the idiopathic or familial condition, but discusses other forms of prolapse where appropriate.

## PATHOLOGY AND PATHOGENESIS

Myxomatous degeneration of the mitral valve leaflets has been demonstrated in mitral valve prolapse of the idiopathic (19, 20, 35, 46–56) and familial type (41), as well as in Marfan's syndrome (22, 54, 57–59) and when prolapse has been associated with secundum atrial septal defect (47). The posterior leaflet is always involved, the anterior leaflet less frequently (47, 53). In a significant percentage of cases the tricuspid valve is involved (47, 60). Involved leaflets (or individual scallops of the posterior leaflet) are voluminous, thickened, and elongated; they present a hooded or domed appearance convex toward the left atrium (Figure 1). The chordae tendineae are usually elongated and thickened (Figure 1), but may be thinned. A significant proportion of cases show ruptured chordae (19, 47, 49, 52, 53, 55, 56). Thrombi on the valve leaflets have been observed either in the acute angle between the prolapsed posterior leaflet and left atrial wall (unpublished observation by the authors), or over cracks in the endothelial surface (47). Jet lesions in the left atrium as well as enlargement of the atrium and left ventricle occur in cases with a significant degree of mitral regurgitation (47). Microscopy of involved leaflets shows replacement of the central fibrous part by metachromatically staining loose myxomatous material, with fibroelastic thickening of the adjacent endocardium (35, 41, 47, 48, 51). These myxomatous changes also occur in the mitral annulus and chordae tendineae, with sclerosis also affecting the latter (47, 48). Although myx-

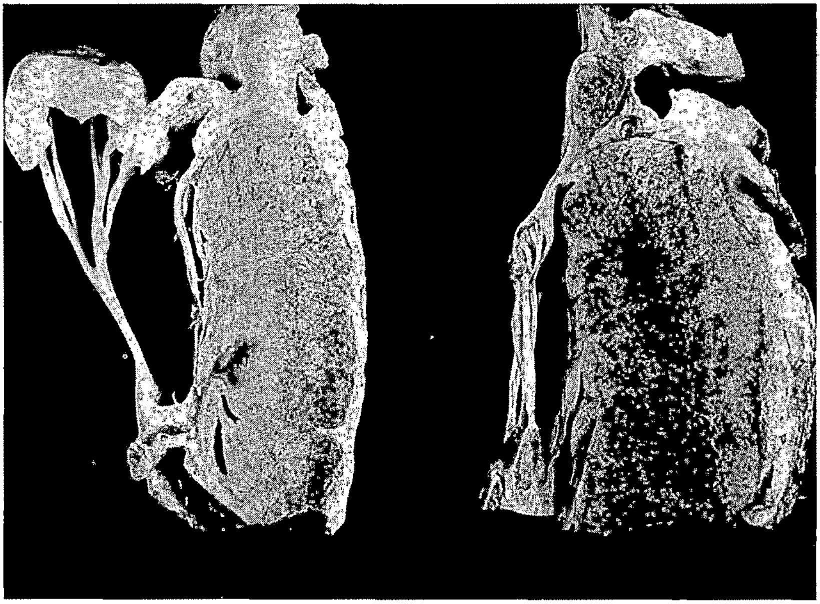

*Figure 1* Comparison of a sectioned middle scallop from a prolapsed posterior mitral valve leaflet (*left*) with that from a patient of the same age with a normal leaflet, but left ventricular hypertrophy (*right*). In the voluminous prolapsed leaflet (*left*), most of the thickening and lengthening of the leaflet has occurred in the rough zone, into which the elongated thickened chordae insert. Scale in centimeters. [Reprinted from *American Heart Journal* (48, p. 539) by permission.]

omatous degeneration appears to be the common pathologic basis for mitral valve prolapse, in our present state of knowledge it is perhaps best to think of this type of degeneration as a nonspecific reaction that may be familial or a forme fruste of Marfan's syndrome in some cases, but in others it may be a result of the abnormal stresses and strains to which the mitral valve is exposed. Indeed, up to 80% of patients with mitral valve prolapse have abnormal left ventricular function (42, 61–68), causing some authors to suggest that the prolapse may be secondary to an ill-understood form of cardiomyopathy (68). The valves of patients with the "click-murmur" syndrome and a history of rheumatic fever, or in association with hypertrophic obstructive cardiomyopathy, have not, to our knowledge, been examined pathologically. Mitral valve prolapse can clearly result from coronary artery disease (21, 25–29), and in four such patients postmortem examination demonstrated normal mitral leaflets (29). In three there was a healed diaphragmatic myocardial infarct with fibrosis of the posteromedial papillary muscle, while in the fourth there was a healed anterolateral infarct with fibrosis of the anterolateral papillary muscle (29). In such instances prolapse is thought to be a form of papillary muscle dysfunction. However, myxomatous degeneration of the mitral valve may coexist with occlusive coronary artery disease (unpublished observation by the authors).

## ANATOMIC-ANGIOGRAPHIC CORRELATIONS

Pertinent to mitral valve prolapse and to its angiographic recognition is a recent redefinition of mitral valve anatomy (Figure 2) (49, 69). Both anterior and posterior leaflets have a rough zone at their distal free edge where rough zone chordae tendineae attach. Proximal to this is a clear zone to which no chordae attach. The two leaflets are separated from one another by commissures, into which fan-shaped commissural chordae insert. In 92% of normal human mitral valves the posterior leaflet has a triscalloped structure with a large middle scallop and smaller lateral scallops, termed the posteromedial commissural scallop (next to the posteromedial commissure) and the anterolateral commissural scallop (next to the anterolateral commissure). Fan-shaped chordae tendineae insert into clefts between the scallops of the posterior leaflet. This leaflet has a greater length of insertion into the mitral annulus than does the anterior leaflet, and has basal or "third order" chordae inserting into its base (Figure 2) (49, 69).

Many authors have described the angiographic appearance of posterior and anterior mitral leaflet prolapse (11, 18, 20, 23, 35, 36, 38, 48, 53, 61, 62, 70–74). Our own interpretation of the angiographic appearance of prolapse of the posterior leaflet is based on the redefined anatomy of this leaflet (Figures 2 and 3). Thus it is now possible to recognize angiographically prolapse of the individual scallops of

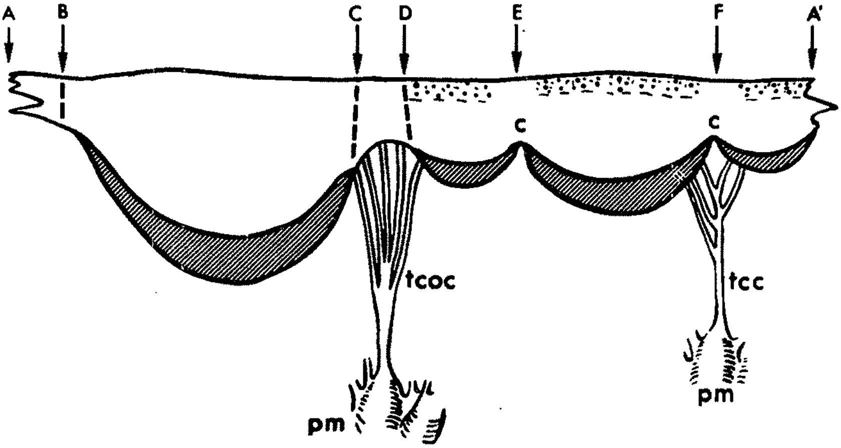

*Figure 2* Diagrammatic representation of the normal mitral valve with typical commissural and cleft chordae tendineae attached. *A-B* = anterolateral commissure; *B-C* = anterior leaflet; *C-D* = posteromedial commissure; *D-A'* = posterior leaflet; *D-E* = posteromedial commissural scallop; *E-F* = middle scallop; *F-A'* = anterolateral commissural scallop; *c* = cleft; *pm* = papillary muscle; *tcoc* = typical commissural chorda; *tcc* = typical cleft chorda. Basal or "third order" chordae insert into the basal zone (*broken line*) of the posterior leaflet. Rough zone chordae of both leaflets insert into the rough zones of these leaflets (*cross-hatched areas*). The intervening areas of the leaflets are the clear zone, to which no chordae attach. [Reprinted from *Circulation* (49, p. 459) by permission.]

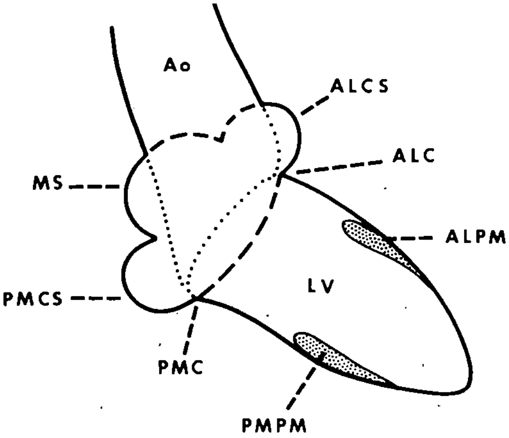

*Figure 3* Line diagram of a left ventriculogram in the right anterior oblique projection. The upper continuous and barred line, extending from the posteromedial (*PMC*) to the anterolateral commissure (*ALC*), outlines the prolapsed scallops of the posterior leaflet of the mitral valve. *PMCS* = posteromedial commissural scallop; *MS* = middle scallop; *ALCS* = anterolateral commissural scallop. For orientation, the locations of the posteromedial papillary muscle (*PMPM*) and anterolateral papillary muscle (*ALPM*) have been outlined in the left ventricle (*LV*). Their tips usually point toward the respective commissures. Also included is the location of the normal mitral valve ring, shown by the lower dotted and barred lines forming a circle. The barred line represents the posterior mitral ring. Note that the anterior part of the mitral ring (*dotted line*) has a common attachment with the aortic root (*AO*). [Reprinted from *Circulation* (53, p. 514) by permission.]

the posterior leaflet of the mitral valve (Figure 3) (48, 53). In the right anterior oblique left ventricular cineangiogram, prolapse of the middle scallop occurs centrally overlying the left atrium, whereas prolapse of the posteromedial commissural scallop causes an inferior bulge, and prolapse of the anterolateral commissural scallop causes a superior bulge (Figure 3). Jeresaty (73), who has based many of his observations on these anatomic findings, believes that when the superior bulge is large it represents prolapse of the anterior leaflet rather than the anterolateral commissural scallop of the posterior leaflet, a point with which we do not agree (74). In our opinion, the size of the superior bulge would be determined by the degree of prolapse of the anterolateral commissural scallop (74). Prolapse of the posterior leaflet occurs in virtually every case (18, 35, 36, 38, 53, 74). Anterior leaflet prolapse is less common (35, 53). Criteria for the angiographic diagnosis of anterior leaflet prolapse have been described (74). In the right anterior oblique left ventricular cineangiogram, prolapse of the anterior leaflet often overlaps prolapse of the middle scallop of the posterior leaflet, but the two can be distinguished by the presence of discontinuity in the angiographic outline, as well as by temporal dissociation (74). The left anterior oblique left ventricular cineangiogram is particularly valuable in documenting anterior leaflet prolapse (74), where its appearance is distinct from the cauliflower-like appearance of posterior leaflet prolapse in this view (38). Using these criteria, we found that of 55 patients with angiographically proven prolapse, 16 had anterior as well as posterior leaflet prolapse. Pathologic proof of combined leaflet prolapse was demonstrated in 5 of these patients (unpublished observations by the authors).

## THE CLINICAL SYNDROME

### *History*

Idiopathic or familial mitral valve prolapse occurs predominantly in females and at virtually any age (17, 26, 35). These patients commonly present with palpitations, presyncope, syncope, chest pain, dyspnea, and/or fatigue (17–19, 35, 38). The palpitations, presyncope, and syncope can usually be attributed to atrial and/or ventricular arrhythmias (17, 35). Sudden death has been reported in a number of instances and is presumed due to ventricular arrhythmias (17, 19, 40, 41, 48, 68). We have recorded seven instances of ventricular fibrillation as the cause of syncope in these patients. Spontaneous defibrillation was observed in three of four patients with recurrent syncope (Rakowski, H., Wald, R., Waxman, M. B., and Wigle, E. D., unpublished observations). The chest pain may be of several different types: (*a*) typical angina of effort, (*b*) unstable angina pectoris, (*c*) prolonged episodes of atypical anterior chest pain lasting for hours or days and not resembling *a* or *b*, or (*d*) sharp stabbing left chest pain suggestive of cardiac neurosis. Increased arterial systolic pressure can induce the atypical episodes of chest pain, suggesting an imbalance between oxygen supply and demand as the basis for this common symptom (75). Dyspnea, fatigue, and evidence of left and right heart failure may result from left ventricular dysfunction (68) or from hemodynamically significant mitral regurgitation (53). More commonly the dyspnea and fatigue cannot be explained on the basis of hemodynamic abnormalities, nor does controlled exercise testing provide objective evidence to explain these symptoms (65). Cerebral ischemic episodes (76) and neurologic deficits (36, 76) may be due to emboli (76) arising from thrombi on the mitral leaflets (47). A number of patients with idiopathic prolapse are asymptomatic and come to cardiological attention because of abnormal findings on physical examination.

### *Physical Examination*

EXTRACARDIAC In some series (42–44, 65), up to 70% of patients with mitral valve prolapse have thoracic skeletal abnormalities suggestive of a forme fruste of Marfan's syndrome: pectus excavatum, straight back syndrome, scoliosis, or narrow anteroposterior diameter of the chest. Many patients, however, have no such stigmata.

CARDIAC In the typical case, the heart is not enlarged and abnormalities on physical examination are restricted to auscultation at the cardiac apex, where a mid-systolic click and/or late systolic murmur are heard. The clicks may be multiple (15, 17, 36, 38, 77) and may occur in early (17, 78, 79), mid (15, 17, 36) or late (17, 19, 38) systole. They usually initiate the murmur but may occur in the middle or towards the end of it. Because of the variable timing of the clicks, they should not all be termed mid-systolic, but rather nonejection clicks (19), to distinguish them from the ejection variety. Although the earliest click vibrations occur shortly after the onset of prolapse (80), the loudest vibrations coincide with maximal prolapse of the leaflets (18) and have been ascribed to a tautening of the lax chordae (chordal snap) (15) or to the leaflets themselves (18), similar to an opening snap in mitral stenosis (28). Multiple clicks may be due to asynchronous prolapse of either the leaflets or individual scallops of the posterior leaflet, or to asynchronous chordal tautening (unpublished observations by the authors). The murmur is most commonly late systolic and crescendo, or crescendo-decrescendo, up to and beyond aortic valve closure (17, 19, 24, 37, 77). In cases with more severe degrees of mitral regurgitation the murmur is usually pansystolic, sometimes with late systolic accentuation. Intracardiac phonocardiography has shown that the click and murmur arise in the mitral area and radiate into the left atrium (18, 24, 37, 77). The murmur and click may also be recorded in the inflow tract of the left ventricle (19, 70). A late systolic murmur may have an early systolic component (24). Intraatrial sound recordings, as well as other studies, have demonstrated that precordial "honks" or "whoops" are usually due to mitral valve prolapse (19, 21, 24, 37, 81, 82). Although a systolic retraction coincident with the click of prolapse can be recorded by apexcardiography (83–86), this phenomenon is rarely appreciated on palpitation. A number of patients with angiographically documented prolapse have no abnormalities on physical examination. Some, but not all, will manifest a click and/or murmur with various provocative maneuvers, such as standing (19, 21). This is important to realize because patients may have symptoms attributable to prolapse, but no clinical evidence of it. In such patients echocardiography and/or angiography are required for an accurate diagnosis. It is important to indicate that systolic clicking sounds have been heard in mediastinal emphysema (87) and left-sided pneumothorax (88, 89). Systolic clicks have also been reported in aneurysms of the membranous ventricular septum (90, 91). Whether they can also be attributed to pericardial (11) or pleuropericardial (3, 4) adhesions is in some doubt at this time.

Patients with mitral valve prolapse due to coronary artery disease tend to be older and male; they usually manifest a loud first heart sound and third and fourth heart sounds, in addition to a late systolic murmur, with or without a nonejection click (21, 25–29). Inspiration accentuates these findings (29). In the click-murmur syndrome following mitral commissurotomy, the click occurs in early systole and the apical murmur is longer (19). We have recently noted the preoperative occurrence of prolapse associated with mitral stenosis in five patients. In four the prolapse was visualized angiographically, and in two it was recognized clinically by the presence of a click in one patient, and a pansystolic murmur with late systolic accentuation in the second (unpublished observations by the authors). The occurrence of mitral valve prolapse in association with secundum atrial septal defect is well recognized (17, 26, 31–35, 47) and occurs in about 40% of cases (34). Half of these cases had clinically silent prolapse and in the remainder an apical pansystolic murmur was the commonest finding indicating prolapse (34). This combination must be distinguished from primum atrial septal defect, in which left axis deviation in the electrocardiogram is virtually diagnostic.

VARIABILITY OF SIGNS Tremendous interest has centered on maneuvers that alter the timing and intensity of the click and murmur (6, 16–21, 61, 80, 92–94). Amyl nitrite inhalation causes the click to move earlier in systole and the murmur to lengthen, sometimes becoming pansystolic (16, 17, 19, 20, 80, 92). Soon after inhalation, the murmur becomes fainter (16, 17), but later it becomes louder (16). The strain period of the Valsalva maneuver also causes the click and murmur to move to early systole (16, 19, 92). Sitting or standing causes a click, murmur, or honk to appear when not present in the supine position, and causes clicks and murmurs to occur earlier in systole (19, 21, 61, 92). Murmurs also become louder (19, 21, 61, 92). Squatting reverses the changes brought about by standing (21). Right atrial pacing tends to move the clicks and murmurs to early systole (94). Infusion of pressor amines either does not change the timing of the click, or causes it to disappear while accentuating the late systolic murmur (16, 19, 93). Many of these variations have been attributed to changes in left ventricular volume in the presence of elongated chordae tendineae (16, 19–21, 80, 93). Thus when left ventricular volume decreases, the chordae are relatively longer and permit earlier and greater prolapse to occur, accounting for the altered clinical findings. Cineangiography has demonstrated a greater degree of prolapse following amyl nitrite inhalation (20) and in the 45° head-up tilt position (93). A recent suggestion is that changes in left ventricular contractility or ejection rate, rather than altered ventricular volume, account for the variability in the degree of prolapse and in the accompanying clinical signs (94).

### *Laboratory Investigation*

ELECTROCARDIOGRAM AND VECTORCARDIOGRAM Many patients with mitral valve prolapse have a normal electrocardiogram and vectorcardiogram, but a very significant percentage manifest definite abnormalities in the absence of coronary artery disease (17, 26, 35, 36, 38, 40, 61, 65, 68, 71, 79, 92; 95–98). The most characteristic change is ST segment change, and T-wave inversion in leads II, III, AVF, V5, and V6, suggesting inferolateral ischemia (17, 36). These abnormalities are sometimes accompanied by tall, peaked T-waves in leads V2 and V3. Others have reported T-wave inversions in the anterior (precordial) leads (35, 68, 79, 97). Q-waves suggestive of both inferior (36, 48, 68, 92, 98) and anterior (68, 95, 98) myocardial infarction have been observed. The explanation of these abnormalities is not known but myocardial and/or papillary muscle ischemia has been suggested (26). Papillary muscle fibrosis has been demonstrated in one patient showing these electrocardiographic abnormalities (48). Prolonged Q-T intervals (17, 34, 65), and prominent U-waves (17, 65) have also been noted. Nearly every conceivable arrhythmia and conduction defect has been recorded in these patients, and exercise electrocardiograms have revealed ischemic ST segment depressions in a significant number (68, 79, 92, 95, 96). Left ventricular hypertrophy has been reported both in the presence of significant mitral regurgitation and in its absence (38, 53, 65, 95).

ARRHYTHMIAS Cardiac rhythm disturbances can account both for the symptoms of palpitations, presyncope, and syncope, and for the occurrence of sudden death in this syndrome. Atrial, junctional, and ventricular arrhythmias of most types have been clearly documented at rest (17, 26, 35, 65, 68, 95, 96, 99, 100), during exercise (26, 65, 68, 95, 96, 99, 100), in the post-exercise period (26, 65, 95, 96, 100), and by ambulatory monitoring (35, 79, 99, 100). Criley has suggested that the atrial arrhythmias may arise from systolic stretch of the valve leaflets and that the ventricular arrhythmias may result from the "diastolic dumping" of the blood held under the prolapsed leaflets in systole (100). The prolonged Q-T interval may predispose to the occurrence of ventricular arrhythmias (17), but the latter may occur in the absence of Q-T prolongation (26, 95). The fact that the click often coincides with the peak of the T-wave indicates that the mechanical force of the prolapse itself might incite a ventricular arrhythmia. Serious ventricular arrhythmias occur in the absence of resting electrocardiographic abnormalities (26). Bradyarrhythmias, including all degrees of heart block, occur in these patients but are relatively rare (68, 100).

ECHOCARDIOGRAPHY Two basic types of echocardiographic abnormalities have been described in patients with mitral valve prolapse (101–105): (*a*) mid-systolic "buckling" associated with mid-systolic prolapse, and (*b*) pansystolic "hammocking" associated with pansystolic prolapse. Another echocardiographic abnormality, "pansystolic collapse," has been described (105). In essence, all of these findings record the prolapse of one or both mitral leaflets into the left atrium during systole. Single-beam echocardiography probably strikes the middle scallop of the posterior leaflet, as well as the anterior leaflet. Prolapse of the posteromedial and/or anterolateral scallop could therefore be missed (unpublished observations by the authors). For this and other reasons, left ventricular cineangiography remains the standard for the definitive diagnosis of mitral valve prolapse, even though the echocardiographic diagnosis of prolapse represents a tremendous advance.

RADIOLOGICAL EXAMINATION Frontal and lateral chest X rays usually reveal a normal heart size and, frequently, thoracic skeletal abnormalities (42–44). Left atrial and ventricular enlargement occurs in the presence of hemodynamically significant mitral regurgitation (53, 68). Left ventricular cineangiography permits assessment of the anatomy of the leaflet prolapse (see section on anatomic-angiographic correlations), the degree of mitral regurgitation, and left ventricular function. The mitral regurgitation may vary from being absent to severe and may begin at the onset of systole in pansystolic prolapse, or in mid-systole in mid-systolic prolapse. Various abnormalities of left ventricular function in systole and diastole have been described in up to 82% of patients studied (42, 61–68). The commonest abnormalities in systole are: (*a*) an inferior contraction ring (42, 62, 65), which may occur alone or be associated with increased (42) or decreased (42, 65, 67) contraction of the anterior wall; (*b*) decreased contraction or expansion of the anterior wall alone (65, 67, 68); (*c*) generalized hypokinesis (68); (*d*) hypokinesis of the inferobasal segment (42, 66), with poor contraction (66) or expansion of the mitral annulus (63, 66), sometimes associated with basal displacement of the tip of the posteromedial papillary muscle (66); (*e*) decreased shortening of the long axis of the ventricle (42, 65, 68); or (*f*) cavity obliteration (42, 64). Early diastolic relaxation, particularly of the anterior wall, has been a frequent finding (42, 65, 67, 68). The ejection fraction may be normal, increased, or decreased, depending on the degree of left ventricular dysfunction and mitral regurgitation (68). The evidence of left ventricular dysfunction has caused some workers to suggest that patients with mitral prolapse may have a cardiomyopathy (68). However, left ventricular function has been normal in some patients with documented prolapse (34, 42, 65). Tricuspid valve prolapse has been observed angiographically in up to 54% of patients with mitral prolapse (42, 106). Selective coronary arteriograms are normal in individuals with idiopathic or familial prolapse, but may demonstrate an unduly tortuous or "corkscrew" appearance (42, 79). Coronary arteriograms in patients with prolapse due to coronary artery disease usually demonstrate severe obstructive lesions (29).

HEMODYNAMICS Intracardiac pressures and flow are usually normal in the absence of significant left ventricular dysfunction or mitral regurgitation. In the presence of either, however, left ventricular end-diastolic pressure may rise and cardiac index may be reduced (68). With significant mitral regurgitation the left atrial V-wave may rise significantly (53).

## COMPLICATIONS

A number of life-threatening complications may occur in patients with mitral valve prolapse. Those with serious ventricular arrhythmias (particularly ventricular tachycardia and fibrillation) are prone to recurrent presyncope, syncope, and sudden death. Infective endocarditis has been well documented (23, 40, 45, 47, 54, 56, 107, 108). Chordae tendineae may rupture from the myxomatous degeneration, or from infective endocarditis, producing severe mitral regurgitation, left and right heart failure, and the need for mitral valve replacement. Emboli can arise from the bland thrombi that may form on the valves of these patients.

## NATURAL HISTORY AND PROGNOSIS

Several reports have suggested that the clinical course of patients with mitral valve prolapse is usually benign (56, 109). In one report (109), 62 patients were followed an average of 14 years. Forty-one were unchanged, ten had slight deterioration, and the remainder either developed infective endocarditis, severe mitral regurgitation, or were lost to follow-up. Patients with cardiac enlargement, abnormal electrocardiograms, and chest pain resembling angina were excluded from this study (109). In contrast with this relatively benign course are the many reports of sudden death (17, 19, 40, 41, 48, 68), the onset of heart failure from mitral regurgitation requiring valve replacement (50, 56), the occurrence of infective endocarditis (23, 40, 45, 47, 54, 56, 107, 108), and reports of continued disability with nonlethal arrhythmias, chest pain, dyspnea, and fatigue. Obviously, the natural history can vary tremendously, and this presents a challenge in medical care today. It is important not to induce a cardiac neurosis in these patients with too much concern, while at the same time dealing appropriately with the complications known to occur, particularly life-threatening ventricular arrhythmias.

## MANAGEMENT

To prevent infective endocarditis, patients with mitral valve prolapse require antibiotic prophylaxis at appropriate times. The treatment of endocarditis should be according to the antibiotic sensitivities of the infective agent. The development of heart failure from significant mitral regurgitation requires appropriate antifailure therapy initially and may require subsequent mitral valve replacement. A number of reports have stressed a high incidence of valve dehiscence in patients treated surgically (45, 55, 56, 110), presumably because of myxomatous change in the annulus. The insertion of deep, interrupted sutures has been recommended to avoid this complication (56). The greatest problem in management is the control of life-threatening ventricular arrhythmias. $\beta$-Adrenergic blocking agents appear to be the drugs of choice, but are not universally efficacious, requiring the additional or sole use of other antiarrhythmic agents. It has been suggested that the mitral valve be replaced to control particularly persistent ventricular arrhythmias, but the long-term value of this aggressive form of therapy is not known (111). Five years ago we replaced the mitral valve in one patient with recurrent ventricular fibrillation who also had severe mitral regurgitation. Ventricular fibrillation has not recurred, but the patient has required continued antiarrhythmic therapy (A. G. Adelman, unpublished observation). $\beta$-Adrenergic blocking agents have also been of value in the management of patients with angina or atypical chest pain.

## SUMMARY

Mitral valve prolapse is a condition that is being recognized with increased frequency. It is not known whether its incidence is increasing, or whether we are better able to diagnose it today. In the idiopathic or familial variety, the mitral valve pathology is almost always that of myxomatous degeneration. Some authors have suggested the presence of a cardiomyopathy because of significant left ventricular dysfunction in many cases. Idiopathic prolapse occurs predominantly in females, often at a young age, and may be associated with chest pain, dyspnea, fatigue, presyncope, syncope, and/or sudden death. The clinical findings are variable and typically consist of a nonejection click and/or late systolic murmur, heard best at the cardiac apex. Diagnosis can be confirmed by echocardiography and/or ventricular cineangiography, the latter permitting accurate recognition of the anatomy of the prolapsed leaflets. The complications of infective endocarditis, severe mitral insufficiency, and life-threatening ventricular arrhythmias represent the major problems of management. It is important to distinguish the idiopathic form of mitral valve prolapse from that due to coronary artery disease and to realize that mitral valve prolapse may occur in Marfan's syndrome, Turner's syndrome, or in association with secundum atrial septal defect or ruptured chordae tendineae. Typical clicks and/or murmurs have also been described in patients with a history of rheumatic fever and in hypertrophic cardiomyopathy. Although much descriptive knowledge has accumulated over the past 15 years, many unanswered questions remain regarding the idiopathic type of prolapse. What is the nature and cause(s) of myxomatous degeneration? What is the relation of the valve pathology to the left ventricular dysfunction? What is the relation of both of these factors to disabling chest pain, electrocardiographic changes, and life-threatening arrhythmias? Hopefully, answers to these and other important questions regarding mitral valve prolapse will be forthcoming.

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# THE EFFECTS OF CALCIUM AND PHOSPHORUS IN HEMODIALYSIS

❖7191

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## INTRODUCTION

An effort is made in this review to examine the effects of calcium (Ca) and phosphorus (inorganic phosphate, $P_i$) in chronic renal failure, with special reference to hemodialysis. Although there are many facets of these effects, involving a variety of organ systems, the subject matter is limited to those involving the skeleton and parathyroid glands, in order to provide a cohesive description. It is probable that one of the major limitations to the successful long-term maintenance of patients with end-stage renal failure by hemodialysis has been the frequent development of disabling osteodystrophy, comprising at least osteomalacia and osteitis fibrosa (1–4). The clinical and roentgenographic frequency of osteodystrophy may approach 100% by the third year of continued hemodialysis (5). Until recently, it has not been clear whether such a high rate was due to the dialysis regimen per se or simply to inevitable progression of a relentless process permitted to become manifest by prolongation of life (4). It is now established, however, that some or all dialytic bone disease may be reversible or preventable by appropriate correction of those factors identified as etiologic. The effects of Ca and $P_i$ are discussed, therefore, in relation to a pathogenetic schema for the development of osteodystrophy. A simplified schema of the interactions to be discussed is shown in Figure 1. Although it has been long known that Ca and $P_i$ metabolisms are deranged in patients with chronic renal failure, it is only within the last decade that new analytic techniques have permitted a reasonable description of the manner in which these abnormalities occur and interact. The precise mechanisms by which these factors act are still to be elucidated, but the schema provides a suitable framework for further study.

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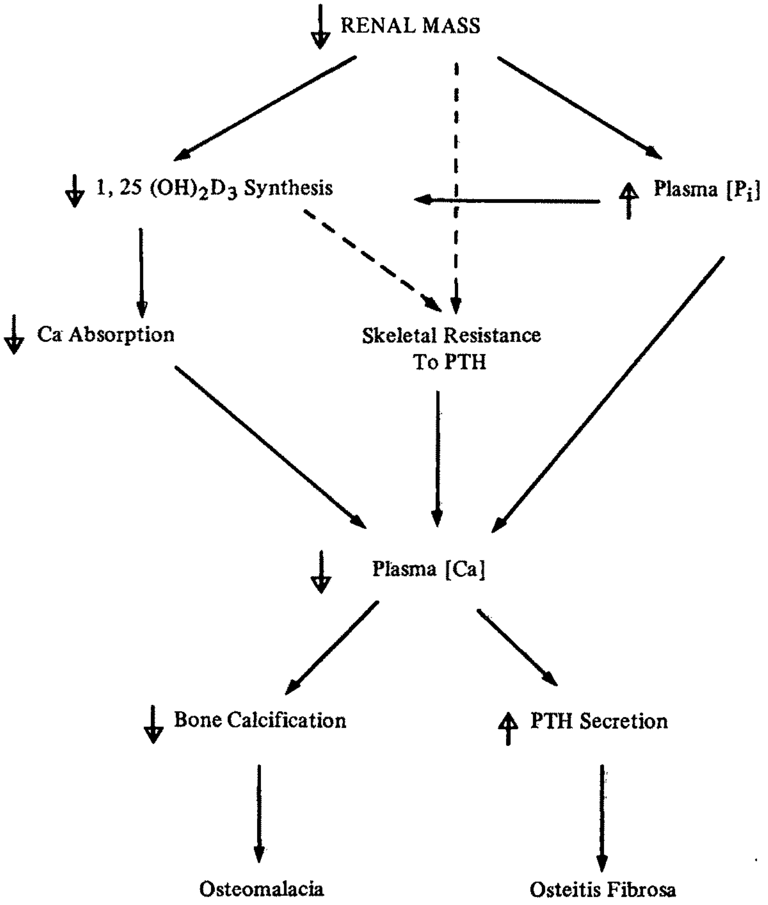

*Figure 1* Pathogenesis of renal osteodystrophy. The sequence of disturbances in calcium and phosphorus metabolism in renal insufficiency leads inevitably to the development of osteitis fibrosa and osteomalacia, the two major components of renal osteodystrophy. Although other, as yet unidentified, factors may be involved in development of osteodystrophy, the sequence shown represents a summary of our current understanding.

## PHOSPHORUS RETENTION AND HYPERPARATHYROIDISM

It is generally considered probable that the two main initiating factors of the overall sequence leading to hyperparathyroidism and osteodystrophy are (*a*) decreased ability of the kidneys to excrete $P_i$ normally and (*b*) decreased synthesis by the kidneys of the major active metabolite of vitamin $D_3$, 1,25-dihydroxy-vitamin $D_3$ [$1,25(OH)_2D_3$]. In 1972, Bricker proposed a "trade-off hypothesis" (6) as an explanation for many of the features of renal failure. The basic concept behind this hypothesis is that many of the abnormalities caused by renal failure lead to activation of compensatory mechanisms that tend to correct the abnormalities at the expense of producing other, less immediately life-threatening, complications. This trade-off hypothesis has been most eloquently elucidated by Bricker, Slatopolsky, and others (7–19) in relation to $P_i$ retention, secondary hyperparathyroidism, and osteitis fibrosa. In this case, the loss of functioning nephrons leads inevitably to at least transitory $P_i$ retention, activating the sequence illustrated in Figure 1. Only through an increase in secretion of parathyroid hormone (PTH) is the plasma concentration of Ca able to be maintained, by the combined effects of increased bone resorption (mobilizing Ca) and decreased renal tubular reabsorption of $P_i$ (increasing the excretion of retained $P_i$). Increased secretion of PTH must be sustained in order to offset the $P_i$ retention by the decreased number of functioning nephrons. With each new "wave" of nephron loss, secretion of PTH must increase above its previous level, leading ultimately to hyperparathyroidism and osteitis fibrosa. In the early stages of nephron loss, plasma concentrations of Ca and $P_i$ may be maintained within the normal range by this mechanism, which fails to be completely compensatory only when glomerular filtration falls below the level at which filtered load of $P_i$ equals the amount of $P_i$ absorbed from the intestine. At this point, the filtered load of $P_i$ can be increased only by an increase in plasma $[P_i]$, leading to the hyperphosphatemia and hypocalcemia characteristic of renal failure with glomerular filtration rates below 25–30 ml/min (6, 7).

There is a substantial amount of experimental support for the role of $P_i$ retention as a prime factor in the development of secondary hyperparathyroidism. One recent report, however, has raised a question about the validity of the concept that the PTH response is necessary to increase the excretion of retained $P_i$. Swenson and colleagues (20) demonstrated that thyroparathyroidectomized dogs were able to increase the fractional excretion of $P_i$ (phosphorus clearance/inulin clearance) and to maintain an unchanged serum $[P_i]$ during surgical reduction in renal mass. These data suggest that there is an alternate mechanism for controlling renal tubular reabsorption of $P_i$ besides PTH, but does not necessarily disprove the role of PTH as depicted in Figure 1.

Several lines of evidence have been developed to support this hypothetical sequence. First, it has been reported from two laboratories that plasma concentrations of immunoreactive PTH are increased in renal failure patients with normal plasma [Ca] and $[P_i]$ at glomerular filtration rates as high as 40–60 ml/min (21, 22). A direct test of the hypothesis has been made in dogs subjected to partial nephrectomy. In these animals, dietary $P_i$ was reduced in proportion to the measured decrease in glomerular filtration rate after surgery, and it was demonstrated that plasma concentrations of Ca, $P_i$, and immunoreactive PTH remained unchanged for the duration of study (9). In similarly nephrectomized animals treated without control of dietary $P_i$, the expected hyperparathyroidism appeared. Although it may appear that these studies negate the importance of the vitamin D sequence on the left of Figure 1, this most likely can be accounted for by the relatively high dietary Ca used in these animals (see below).

Additional evidence has been developed in several laboratories by observation of the effects of therapeutic intervention on plasma concentration of immunoreactive PTH(iPTH) and on roentgenographic and histologic examination of the skeleton (13, 14, 17–19). In one series of studies (18, 19) it was shown that plasma iPTH was positively correlated with plasma [$P_i$], both in long-term serial observations on individual patients and in single observations on a large number of patients. In addition, this was found to be the case during acute perturbations (2–3 weeks) of plasma [$P_i$] by the use of oral aluminum hydroxide and carbonate preparations to bind $P_i$ in the intestinal lumen and thus prevent its absorption (13). Berlyne has added further support for the importance of $P_i$ restriction with his observation that renal osteodystrophy is rare in Israel, where the dietary intake of $P_i$ is habitually about 25% lower than that in the United Kingdom (16). It has been demonstrated by neutron activation analysis that patients with severe renal insufficiency have a total body $P_i$/Ca ratio that is significantly increased above normal, principally due to $P_i$ retention (23). In these same patients, total body Ca was inversely related to the calculated size of the "exchangeable" Ca compartment (not anatomically defined) and to the calculated rate of Ca accretion. The authors suggest the possibility that the increase in exchangeable Ca in their patients with hyperphosphatemia may reflect an increase in soft tissue Ca. Other studies of calcification in the skin, cornea, and conjunctiva are in accord with this suggestion, since such calcification generally seems to be related to high plasma [$P_i$] (24, 25). Control of plasma [$P_i$] by use of oral $P_i$-binding aluminum compounds is often followed by a reduction in these soft-tissue deposits of Ca (24, 26).

Although considerable evidence has been accumulated for the importance of preventing or correcting hyperphosphatemia, it is equally important to recognize the danger of excessive $P_i$ depletion (27–31). Both Ca and $P_i$ are required for calcification to proceed normally, and deficiency of either apparently can lead to rickets or osteomalacia (32). Several patients have been reported in whom excessive $P_i$ depletion by oral $P_i$-binding aluminum compounds led to hypophosphatemia and osteomalacia (30, 31).

## VITAMIN D RESISTANCE AND CALCIUM DEFICIENCY

All of the factors responsible for the development of osteomalacia in patients with chronic renal failure may not be known, although it seems probable that both hypocalcemia and vitamin D "resistance" play important roles (33–37). Hypocalcemia per se leads to defective calcification of newly formed osteoid (38), so that the various factors that cause hypocalcemia are probably responsible in part for the excessive amount of uncalcified osteoid and decreased calcification front that are features of renal osteodystrophy. However, the higher frequency of a major osteomalacic component in England and France than in the United States seems to be related to the relative vitamin D deficiency in those countries, due both to decreased intake and to decreased endogenous synthesis of vitamin D as a result of limited exposure to sunlight (37). Thus, patients with reduced vitamin D stores would be expected to be more susceptible to other factors that may be etiologic for osteomalacia.

In addition to vitamin D deficiency, patients with renal failure are "vitamin D resistant," i.e. they fail to respond to large doses of vitamin D with the expected increase in plasma [Ca]. Reports from several laboratories (39–41) have placed this resistance on a firm biochemical foundation by demonstrating that the kidney alone is responsible for synthesis of 1,25-dihydroxy-vitamin $D_3$ [$1,25(OH)_2D_3$], the most potent known metabolite of vitamin $D_3$ in terms of stimulating intestinal Ca absorption. As would be anticipated, this metabolite does not appear to be synthesized in measurable amounts by the patient with renal failure (42). In addition to loss of cells producing the needed enzyme, there is evidence from experimental animals that $P_i$ retention may inhibit synthesis even in the absence of renal insufficiency (43). Thus, before renal damage has progressed to the point where the hydroxylase is actually deficient, its activity may be significantly reduced by $P_i$ retention. Some authors have found that intestinal Ca absorption tends to return toward normal as secondary hyperparathyroidism becomes severe (44, 45). It is possible that such an effect could be due to stimulation of $1,25(OH)_2D_3$ synthesis by the increased circulating concentrations of PTH (46).

Both in patients with acute and chronic renal insufficiency, the skeleton has been shown to be resistant to stimulation of resorption by PTH (47, 48). In experimental animals, this resistance can be demonstrated shortly after the production of renal failure (48). The mechanism by which this effect occurs is unknown, but it may relate to the apparent deficiency of $1,25(OH)_2D_3$ (48–50). It is possible that this represents a protective mechanism so that the skeleton will not be too rapidly resorbed by the large amounts of PTH secreted.

Probably the only evidence supporting a direct role of vitamin D in bone calcification has been that of Eastwood and his colleagues (51). In this study, patients with renal failure either were treated with vitamin D or had their plasma [Ca] and [$P_i$] maintained within the normal range by Ca supplementation and $P_i$ deprivation. In the latter group, bone biopsy disclosed the appearance of punctate calcification in the osteoid, but no calcification front; in the former group, a calcification front characteristic of normal calcification was observed. Thus, these data suggest the need for vitamin D or one of its metabolites for calcification to proceed normally.

Presumably as a result of $1,25(OH)_2D_3$ deficiency, patients with chronic renal failure do not absorb Ca normally from the upper small intestine (52–58). An interesting facet of this problem has been described recently by Oettinger and his colleagues (59). They observed that intestinal Ca absorption decreased significantly in chronically hemodialyzed patients after bilateral nephrectomy, suggesting that even kidneys without measurable excretory function may still retain some ability to synthesize $1,25(OH)_2D_3$. Nonetheless, considerable data indicate that $1,25(OH)_2D_3$ synthesis is deficient even before nephrectomy (42). Thus, with $1,25(OH)_2D_3$-dependent Ca absorption being less than normal and endogenous secretion of Ca into the lower intestine being apparently normal or increased (45, 57), these patients characteristically lose more Ca in their feces than they ingest in their diets and are therefore in chronic negative Ca balance (1, 2, 34, 45, 56). Since Ca absorption is normally a combination of active and passive (diffusional) mechanisms, however, it is possible to compensate for the deficient active portion by increasing that due to diffusion (55, 60, 61). This has been accomplished successfully by the oral administration of relatively large Ca supplements (60, 61) which presumably increase the gradient from intestinal lumen to plasma. These oral supplements thus tend to bypass the $1,25(OH)_2D_3$ deficiency and serve to maintain plasma [Ca] and to suppress plasma [iPTH] (19), as well as to cause punctate calcification within the excessive osteoid (51).

In patients dialyzed against the frequently employed bath concentration of 5.0–5.5 mg/dl (11), even more Ca may be lost (62–74). Several investigators have demonstrated that the most important determinant of Ca flux across the dialyzer is the gradient from plasma dialyzable or ultrafiltrable [Ca] to dialysate [Ca], most of which is dialyzable (62–64). Net Ca flux is in the direction of the Ca gradient (63–70). In general, this means that a dialysate bath [Ca] less than about 6 mg/dl results in net movement of Ca from the plasma to the bath, further exacerbating the negative Ca balance due to poor intestinal Ca absorption. Bath [Ca] higher than about 6 mg/dl, on the other hand, leads to net Ca transfer from the bath to the patient, tending to counteract intestinal Ca losses. Neutron activation analysis has supported these short-term observations by showing that patients dialyzed against bath [Ca] of 8 mg/dl experienced a cumulative gain in total body Ca, while those dialyzed against bath [Ca] of 6 mg/dl experienced a cumulative loss of total body Ca (71–74). A major secondary effect of higher dialysate [Ca] is suppression of plasma values of [iPTH] (12, 13, 18, 19, 75, 76), acutely and chronically, presumably reflecting decreased secretion of PTH. Indirect support for the probability that PTH secretion is inhibited by high dialysate [Ca] comes from two sources. First, intradialysis plasma [Ca] is increased during use of high dialysate [Ca], just as occurs during intravenous Ca infusion. Since such Ca infusions in dialysis patients have been shown to suppress plasma [iPTH] acutely (75–80), one would expect Ca infusion by dialysis to be equally effective. Second, plasma [$P_i$] has been shown to be more easily controlled by oral aluminum hydroxide and carbonate when the bath [Ca] is higher (13). This appears to be due to decreased release of $P_i$ from bone, in turn due to a decrease in PTH-mediated bone resorption.

Just as excessive $P_i$ deprivation can lead to hypophosphatemia and osteomalacia, so overly vigorous Ca replacement by oral supplements or high bath concentrations can lead to potentially hazardous hypercalcemia (2, 49, 81). Mild hypercalcemia (plasma [Ca] less than about 11 mg/dl) is probably inconsequential, but greater elevations may cause nausea and vomiting, and could be dangerous. There appears to be a wide variation among dialysis centers in the quantitative response of patients to Ca replacement, and it may be necessary therefore to individualize such therapy in order to avoid unnecessary complications.

## SUMMARY

Combined use of Ca supplements (orally or by dialysis) to circumvent decreased intestinal Ca absorption and aluminum compounds to reduce plasma [$P_i$] has been demonstrated to decrease plasma [iPTH], to normal or near normal in many patients, and to decrease the percentage of bone surface involved in bone resorption. A highly significant positive relationship between change in plasma [iPTH] and bone resorbing surface has been reported. It seems evident, therefore, that PTH must be a major factor in the bone resorption of chronic renal failure, despite the relative skeletal resistance to PTH. As a result, correction of factors that increase PTH secretion should be considered a prime goal of therapy in these patients. The osteomalacic element of renal osteodystrophy, on the other hand, is only partially controlled by these maneuvers and probably requires the additional use of vitamin D or one of its active metabolites for full correction.

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# PARIETAL CELL VAGOTOMY WITHOUT DRAINAGE FOR THE TREATMENT OF DUODENAL ULCER

❖7192

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## INTRODUCTION

Parietal cell vagotomy (PCV) refers to section of the vagus fibers supplying the fundic gland area of the stomach. PCV is a misnomer, for all elements of the fundic gland area, not just the parietal cells, are deprived of their vagal control. The branches of the vagus nerves supplying the pyloric gland area of the stomach as well as all other intraabdominal organs are preserved. This operation has several other names including proximal gastric vagotomy, highly selective vagotomy, superselective vagotomy, and selective proximal vagotomy, to mention a few. They all refer to the same procedure, but none reflect precisely the operation performed, which is vagotomy of the fundic gland area. Of the names thus far suggested, I prefer PCV because it relates to the aspect of vagotomy one wishes to accomplish.

Griffith & Harkins (1) proposed PCV without a concomitant drainage operation but did not perform their operation on man. It remained for Holle & Hart (2) to introduce the new type of vagotomy, but in combination with pyloroplasty as a treatment for duodenal ulcer. Holle has steadfastly held to his conviction that a drainage procedure was necessary to permit normal gastric emptying if recurrent ulcer was to be prevented. At about the same time, Amdrup (3), working in Griffith's laboratory, became convinced that PCV unaccompanied by a drainage procedure could reasonably be tried in man. She persuaded Amdrup & Jensen (4) of this and they published their initial results in 1970. Johnston & Wilkinson, working independently, published an abstract on the subject in 1969 and their first paper in 1970 (5). When Johnston & Amdrup realized they were working in the same field they arranged to publish much of their data jointly (6), although both men independently have contributed enormously to the development and understanding of the operation.

## OPERATION

The anterior or left vagus trunk divides into the hepatic branches and into the anterior nerve of Latarjet. This nerve parallels the lesser curvature of the stomach and provides branches to the fundic gland area. Near the angularis incisura the anterior nerve of Latarjet divides into several branches that supply the pyloric gland area of the stomach. The posterior or right vagus trunk divides into the celiac branch and the posterior nerve of Latarjet which, like the anterior nerve, supplies vagal fibers to the fundic gland area and then terminates into several branches that supply the pyloric gland area. The principle of the PCV is to transect all the branches of the anterior and posterior nerves of Latarjet between the junction of the fundic with the pyloric gland areas of the stomach and the esophagogastric junction. The esophagus is encircled, and any additional branches of the vagus on the terminal esophagus that supply the fundus are also cut. Thus the celiac, hepatic, and antral branches of the vagus nerves are preserved. The author reperitonealizes the lesser curvature of the stomach by imbricating the anterior and posterior layers of the stomach. This is done to secure hemostasis, discourage vagal reinnervation if this occurs, and to protect against necrosis and fistula formation (7) that might result from inadvertent injury of the gastric wall. Intraoperative evaluation of the completeness of PCV has been tested by various techniques using an intragastric pH probe, but this is not essential for the satisfactory performance of the procedure.

## POTENTIAL ADVANTAGES OF PCV

Diarrhea following gastric surgery is attributed to truncal vagotomy or loss of pyloric function. The cause for vagotomy diarrhea is not known, but has been considered to be due to intestinal hurry or infection resulting from gastric stasis. The diarrhea resulting from the loss of the pylorus is associated with unregulated gastric emptying. It was expected that preservation of the pylorus and antral innervation would reduce dumping as well as diarrhea, and that preservation of the extragastric vagi would reduce the incidence of vagotomy diarrhea. In one study (6), the incidence of dumping and diarrhea were 5% and 5.6%, respectively, which was significantly less than occurred with truncal vagotomy. Most investigations support these findings.

Since PCV is a less extensive operation than the conventional operations and does not require opening of the stomach or the creation of a suture line, the mortality and the immediate postoperative morbidity have been less than with other forms of gastric surgery. The absence of an anastomosis eliminates such complications as afferent and efferent loop syndromes. The mortality in several series of patients has been zero. (D. Johnston, in a personal communication, has indicated that in his survey of 5200 cases operated by 40 different surgeons, the mortality was 0.31%.). Studies in dog suggest that antral innervation (8) and the extragastric vagi (9) are responsible for important mechanisms that inhibit gastric secretion. Whether these findings are also true for man is unknown, but it is possible that PCV may preserve important mechanisms for the inhibition of acid secretion.

## POTENTIAL DISADVANTAGES

Gastric stasis encountered after truncal vagotomy unaccompanied by a drainage procedure caused concern as to whether similar problems would occur after PCV if no drainage procedure was performed. Clinically, gastric retention has not been a problem if the size of the pyloric lumen was adequate and sufficient innervation of the antrum was retained. In dog, extension of the lesser curvature denervation 2 cm distal to the junction of the fundic-pyloric gland areas resulted in significantly slower gastric emptying (10). In man, however, it is questionable whether it is so critical for good gastric emptying that none of the antrum be denervated. Gastric emptying after PCV has interested a number of investigators. Moberg (11) and Clarke & Alexander-Williams (12) found an increased emptying rate during the first period after the ingestion of a meal but not thereafter. Faster early emptying of saline and hypertonic dextrose solutions was noted by Wilbur & Kelly (13), while Hallenbeck (14) found early emptying only with hypertonic dextrose solution. Early emptying after vagotomy has been attributed to increased gastric pressure following the loss of receptive relaxation by the stomach (13, 14). Although gastric emptying after PCV is usually satisfactory, the presence of pyloric stenosis and/or excessive denervation of the antrum may lead to gastric stasis and recurrent ulcer. That this is a possibility is suggested by the fact that half of the recurrent ulcers in some studies are located in the stomach.

Another potential disadvantage of PCV was the retention of an innervated antrum, which theoretically permits an increased release of gastrin in response to vagal and chemical stimulation. The important question was asked by Johnston et al (15), "Should the gastric antrum be vagally denervated if it is well drained and in the acid stream?" Their findings indicated that acid production in response to a test meal was no greater in patients with a vagally innervated antrum than in patients with a vagally denervated antrum when the antrum was maintained in continuity with the fundus. These findings confirmed the early work of Grossman et al (16), who demonstrated that release of gastrin by chemical agents acting locally in the antrum was not dependent upon intact vagal innervation, but contradict the studies of Emas (17), who showed that denervation of the antrum reduced acid secretion from denervated parietal cells when the antrum was stimulated.

Kragelund (18) stated that innervation of the antrum was necessary for an acid response to feeding with meat extract in patients. According to Debas et al (19), antral denervation in dogs decreased the increment of gastrin released following alkaline antral distension but increased acid response to any given increment of gastrin. They suggested that maintenance of antral innervation was beneficial by virtue of an acid inhibitory mechanism. Loss of this inhibitor after antral denervation accounted, in their opinion, for increased acid secretion from denervated fundic mucosa in response to alkaline distension of the antrum. In support of this hypothesis, studies showed that when the antrum was in the acid stream it was protective against ulcer formation (8) and that it afforded greater protection if its vagal nerve supply was left intact (20). It can be reasonably concluded that the gastric antrum need not be vagally denervated in the duodenal ulcer patient provided that it is well drained and is in continuity with the acid stream from the body of the stomach.

## CLINICAL RESULTS OF PARIETAL CELL VAGOTOMY

Although PCV has had a limited trial in the treatment of gastric ulcers and in the treatment of bleeding, perforated, and obstructed ulcers, it has been used extensively in the elective treatment of duodenal ulcer. In a combined report on 300 patients operated in Leeds and Copenhagen, there were 108 patients followed two to four years. This is the largest group of patients followed for the longest time that is available (6).

While this study was not a randomized trial, the patients were thoroughly evaluated and provided valuable data. Three hundred patients were operated on electively for duodenal ulcer without mortality. Gastric atony requiring nasogastric suction was eliminated, and only one patient required reoperation for gastric stasis. Diarrhea occurred in 5% of patients. Dumping occurred in 6% of patients and could be provoked in 47% of patients by the oral administration of glucose. No patient was disabled by either dumping or diarrhea. Other complaints, such as bilious vomiting and flatulence were also less after PCV than after other types of vagotomy with drainage. In the 108 patients followed for two to four years, two gastric ulcers occurred. Two patients were reoperated for suspected but unconfirmed duodenal ulcers. Results were classified as excellent or good in 88% of patients and failures in 5%. The failures included the patients with stasis, recurrent or suspected recurrent ulcers. The results obtained with PCV were better than those obtained with truncal vagotomy and pyloroplasty (TV-P), truncal vagotomy and gastroenterostomy (TV-GE), and selective vagotomy and pyloroplasty (SV-P), granted that the comparisons were not made in a prospective, randomized manner.

In a prospective, randomized study by Kennedy et al (21), PCV without drainage was compared with selective vagotomy and gastroenterostomy (SV-GE), which they considered the best orthodox procedure. There were 50 patients in each group. There was no operative mortality. The average followup was two years with a range of one to four years. Four patients (8%) had mild dumping after PCV, while 14 patients had mild dumping and 4 had severe dumping (37%) after SV-GE. Two patients had mild diarrhea after PCV, and 5 had mild and 1 had severe diarrhea after SV-GE. Miscellaneous symptoms such as nausea and bile vomiting were more frequent after SV-GE than after PCV. The incidence of heartburn and flatulence was the same in both groups. In Kennedy's opinion, improvement in dumping was due to the elimination of the drainage procedure. This was based on the occurrence of dumping in 7 patients, which was severe or moderate in 3 patients following PCV and GE in a separate group of 24 patients. There was one recurrent duodenal ulcer after PCV, and one jejunal ulcer and one suspected duodenal ulcer after SV-GE. The results in the 50 patients after PCV were excellent or good in 48, fair in 1, and a failure (recurrent ulcer) in 1. The results in 49 patients after SV-GE were excellent or good in 37, fair in 9, and a failure in 3.

Another prospective, randomized controlled study of PCV is that reported by Kronborg & Madsen (22). Fifty patients had PCV and 50 patients had SV-P without a mortality. Patients followed one year had significantly less dumping and diarrhea after PCV than after SV-P. There were 11 recurrences or suspected recurrences after PCV and 4 recurrences after SV-P. The reduced number of gastric complaints after

PCV offset the adverse effects of an increased number of recurrent ulcers so that the overall clinical rating after PCV and SV-P were similar.

Ten days after the operations, Hollander tests were performed. In both groups the Hollander test was positive in 58% of patients and in both groups the percentage of patients with a positive Hollander test was the same in the patients with, as in the patients without recurrent ulcer. No correlations could be established to explain the high recurrent ulcer rate after PCV. In Kronborg & Madsen's opinion, (22) the high recurrence rate after PCV was not related to the high proportion (58%) of positive insulin tests, because this figure was not different from the 51% positive insulin tests reported by Amdrup (6). The difference between the two studies, however, lies in the fact that 58% of their Hollander studies were positive within ten days after surgery while in Amdrup's study (6), only 7% of the Hollander tests were positive immediately after operation and the positive responses did not rise to 51% until one year after operation. The significant difference in the incidence of positive insulin tests immediately after operation suggests that there was a basic difference in the type of operation performed by the two groups of investigators. The possibility for many technical variations in the performance of PCV makes the evaluation of results by different individuals who presumably perform the same operation, difficult. The variability in results achieved by different surgeons performing nominally the same operation but which in fact may be different, is likely to lessen the credibility of a potentially useful operation.

There are numerous other valuable reports on the use of PCV without drainage. Duthie (23), in a nonrandomized comparison of 81 TV-P and 44 PCV, concluded that dumping and diarrhea were less and recurrences were fewer after PCV than after TV-P. A difference in the functional rating did not achieve statistical significance, however. Bombeck (24) studied PCV, truncal vagotomy and antrectomy (TV-A), and selective vagotomy and antrectomy (SV-A) in 34 patients. Basal acid output at the end of one year had returned to 80% of the preoperative level after PCV, 14% after TV-A, and 3% after SV-A. The maximal acid output had risen to 75% of the preoperative level after PCV, 14% after TV-A, and 6% after SV-A. The rise in basal and maximal acid output after PCV exceeds that of any other study reported. There was one recurrent and one suspected recurrent ulcer, both after PCV. Initially, Wastell (25) reported on 36 patients assigned randomly and prospectively to PCV with pyloroplasty and PCV without pyloroplasty. Three to 12 months after operation two recurrent and one suspected recurrent ulcer occurred in the group of 16 patients without pyloroplasty. In a later study, Wastell (26) reported two recurrent ulcers in 52 patients with PCV without drainage and in 2 of 48 patients after PCV with drainage. This suggests that the recurrences reported earlier may have occurred during the period that their operative technique was being developed.

## SUMMARY

PCV without drainage for the treatment of duodenal ulcer is a safe procedure. There are fewer gastrointestinal complaints of the type frequently observed after more orthodox gastric surgery. Diarrhea and dumping may be noted after PCV but the incidence of both is less, and when they do occur they are milder and more easily controlled than after other types of operations. The ultimate recurrent ulcer rate remains unresolved because insufficient time for observation and follow-up has elapsed. Since most recurrent ulcers that occur after other gastric procedures present within the first two years after operation, the documentation by several authors of a recurrent ulcer rate of 2–4% in patients followed two to four years after operation suggests that PCV is a highly effective procedure. Yet the high recurrence rates of 22% reported by one group indicates that the operation is not highly effective for everyone. The factors responsible for the discrepancy between the high and low recurrence rates reported by different authors are not evident at this time.

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# IMPLICATIONS OF MASS AUTOMATED INSTRUMENTS ON MEDICAL PRACTICE

❖7193

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## INTRODUCTION

New instruments have invaded the practice of medicine and could be silent, efficient partners if they weren't so numerically prolific. Housed within the laboratory are mechanical giants which can produce mountains of medical data in an average day's operation. For the practicing physician, these analyzers will be encountered when a routine physical examination is performed and blood work is referred for chemical screening. In addition, hospital laboratories are making increased use of large machines and the physician will utilize results from them when orders are placed for an admission screen, organ profile, or diagnostic disease panel.

## TERMINOLOGY

The most common type of mass automated laboratory instrument performs chemistry tests. We limit our discussion to machines that can determine at least twelve tests simultaneously, since this seems to be a natural breakpoint for high volume, automated instruments. Table 1 lists the most common types of instruments on the market today, along with clinically significant variables determining their individual performance. Two classes of machines are found in Table 1 which represent different primary modes of operation.

### *Discrete Sample Analyzer*

A discrete sample analyzer (DSA) takes a specimen and proportions it into separate, discrete analysis chambers which can be processed independently. This is equivalent to performing each test in a separate test tube (1).

### *Continuous Flow Analyzer*

A continuous flow analyzer (CFA) takes the specimen into appropriate tubing and separates it into small segments by bubbles (2). These small segments pass through

**Table 1** High volume chemical analyzers and their associated clinical operating characteristics

| Instrument | Test channels | Tests/hr | Sample size[a] (ml) | Type[b] | Channels calculated[c] | Notation of pathologic results[d] | Stat time[e] (min) | Start up time from complete shutdown (min) |
|---|---|---|---|---|---|---|---|---|
| Acu Chem® | 17 | 1020 | 0.5 | DSA | select | H-(high)<br>L-(low) | 11 | 35 |
| Autochemist® | 24+ | 3240+ | 1.5 | DSA | all | — | 20–30 | 30+ |
| Coulter Chemistry® | 21 | 1260 | 3.0 | DSA | select | no notation | 30 | 20 |
| Hycel 17/Super 17® | 17 | 1020 | 1.2 | DSA | select | bar graphs | 30 | 30 |
| SMA 12/60® | 12 | 720 | 1.0 | CFA | all | bar code | 8 | 20 |
| SMAC® | 20 | 3000 | 0.5 | CFA | all | bar code | 7 | 45 |

[a] Sample size is total volume needed to perform all channels of reporting.

[b] DSA—Discrete sample analyzer; CFA—Continuous flow analyzer.

[c] Some machines output all results no matter what the request and others do selected tests only.

[d] The final report from the machine shows different techniques for delimiting abnormal results.

[e] If a specimen was sent to the laboratory stat, this would be the shortest time for reporting.

®Trade name.

the tubing and stop at various stations for analysis. Since flow is continuous, each specimen follows the prior sample and, in some cases, can be only one bubble apart. This close proximity of one specimen to another produces carry-over, which must be minimized for accurate results (3). The physical problem of carry-over occurs because of inadequate washing of the tubing between specimens. Various techniques have been employed to eliminate carry-over in new CFA instrumentation, yet this problem still exists and must be seriously considered by the clinical laboratory with each day's work as it is evaluated for reporting.

## WHY ARE THESE MACHINES BECOMING SO POPULAR?

Like every aspect of health care, the clinical laboratory is caught in the price squeeze. Labor, materials, and space continue to escalate in cost, yet there is a constant demand for more data for less money. The only successful solution to such pressure has been the introduction of automation and the mass production of work (4).

Over the past five years we have witnessed the growth of automation from a 30–40-result-per-hour machine up to our present 3000-plus-result-per-hour giant (5, 6). Serious cost analysis has shown that labor can be minimized and/or stabilized. In most cases, the machines use less reagents and therefore decrease overhead expenses.

In addition, the quality of data generated can be more precisely controlled over a long period of time by the use of automation (7). Most of our modern instruments respond to internal computer control and carefully evaluate their own level of performance on a second by second basis. Through the use of internal computer systems, these new machines are conveniently interfaced with laboratory and hospital information systems which can then manipulate the data for convenient retrieval (8).

All of these positive features provide increased productivity for the laboratory and a marked decrease in the cost per test for the patient. For the physician, automation provides rapid reporting of results and more data for the dollar.

## ON THE NEGATIVE SIDE

Our new machines can produce an excessive accumulation of data, and many physicians will find that unwanted results are being supplied and charted on the medical record. This data explosion, in some cases, has resulted from the basic machine design which automatically produces all results possible even if only one or two are requested (See Table 1).

Increased result production has also added the burden of reading and interpreting data with the possible loss or misinterpretation of significant information. This is merely an expansion of the heavy clerical load currently experienced in medical practice.

### *Normal Values*

For many of our new instruments we are dealing with new technology and new methodologies. This means that customary normal ranges will most likely be inadequate and require standardization. Unfortunately, these normal ranges may not be adequately established prior to instrument utilization and most will probably require local review and update. It, of course, takes the time and effort in the laboratory to coordinate this kind of information and make it universally available in an easily understandable form (9).

### *Statistical Dilemma*

Statistics plays a major role in defeating some of the advantages obtained by the use of multi-test instruments. As we increase the number of results per specimen, we also encounter a significant statistical error which is cumulative and depends on the total number of tests being performed (10). This has previously been referred to as the "abnormal-normal" phenomenon, in which patients who are healthy and should be classified as such, suddenly become diagnostic problems because of a significant elevation or depression in one or two variables. Table 2 illustrates how this happens and the frequency with which this can be expected (11). Using the statistical formula $P=(1-0.95\ N) \times 100$, where $N$ equals the number of tests performed and $P$ equals the percent probability of the one "abnormal-normal" result, it becomes quite evident that as the number of tests increases it becomes exceedingly easy to find one result outside of normal limits usually defined as plus or minus two standard deviations.

A bit of background may be of some help in describing this particular phenomenon. When normal ranges are established for an individual test, we customarily apply plus or minus two standard deviations to our normal pool, which eliminates about 5% of the normals from consideration (12). This is an arbitrary decision based

**Table 2** The number of tests performed simultaneously versus the probability of finding an "abnormal-normal" result[a]

| Number of tests[a] | Patients having at least one "abnormal-normal" result (%) |
|---|---|
| 1 | 5 |
| 6 | 27 |
| 12 | 46 |
| 13 | 49 |
| 14 | 51 |
| 15 | 54 |
| 16 | 56 |
| 17 | 58 |
| 18 | 60 |
| 19 | 62 |
| 20 | 64 |
| 21 | 66 |
| 22 | 68 |
| 23 | 69 |
| 24 | 71 |

[a] $P = (1 - 0.95\ N) \times 100$, in which $P$ = percentage of "Abnormal-Normal" results, and $N$ = Number of tests.

on the requirement that the test try to distinguish as closely as possible the sector of normal from that of abnormal. That 5% of the normal population being excluded is therefore being misdiagnosed and is, in many cases, put in jeopardy by subsequent testing and further evaluation. As the range of normal is extended to include larger sections of the normal population, some of the abnormals become misclassified and the sensitivity of the test is greatly compromised (13). When this 5% exclusion for 12 to 20 tests is mathematically added, an exceedingly high probability arises that more than one of the tests will be abnormal on a strictly statistical basis, and have nothing to do with the physiology of that particular patient.

Statistical calculations can be performed to show that 95% of all normals can have at least one variable abnormal when 2–7 tests are performed. When 8–16 tests are run, 95% of all normal people can have two variables outside normal limits strictly by chance. For 17–28 tests, this figure climbs to three abnormal tests by chance (14).

### *What Can Be Done?*

When a battery of tests are run and abnormals are found, the simplest solution to resolving abnormal tests, if they are not expected or anticipated, would be to reorder that test in isolation. It would not be appropriate to reorder the battery of tests because, again, you are subjecting the patient to the same statistical problem. It would also not be recommended to go to more definitive procedures until the original test has been confirmed in isolation. This can usually be requested from the laboratory by asking that a reference method be run to validate an elevated result. Almost every laboratory has a secondary means of testing which can be used to confirm a procedure done on a multi-test machine. If the confirmation is elevated or depressed beyond normal ranges, then it certainly is consistent to pursue this particular variable for further evaluation. If it appears to be normal, then the most likely situation is that you are dealing with a statistical error.

### *Human Variability*

As has been recently shown, the laboratory data we are dealing with are of finite value and normal ranges must be interpreted with a great deal of clinical skepticism (15, 16). We are finding more and more variability with each one of the procedures on an individual basis than many workers had previously thought possible. Such variables as time of day, method of collection, diet, environment, and ethnic background have a major role in determining individual limits. These factors are often invisible modifiers which may cause an unnecessary work-up for a perfectly healthy subject. Such problems exist now and will probably continue to exist until we develop a more scientific means of measuring ranges which may be patient specific.

## IN THE FUTURE

The statistical problem of interpretation of normal ranges can be attacked through the use of a mathematical routine called multivariate analysis (17). Instead of considering one test at a time, one considers a whole group or battery of tests simultaneously. Figure 1 shows how this would apply to two enzyme variables and gives an illustration of the errors that we are currently making when we interpret our normal ranges (18). The small *x*'s represent patient pairs of laboratory data for enzymes: LDH-total and LDH-heat stable. The square represents a univariate (one variable at a time) interpretation of normal, which is consistent with our present manual mode of interpretation. The ellipse represents the true statistically determined normal for these two variables taken simultaneously. As you can see, the ellipse much more precisely defines the range of normal and could accurately include 95% of the population. The square is not nearly as exact and misclassifies a number of individuals.

Figure 2 illustrates a normal range ellipsoid for three variables: serum glutamic oxaloacetic transaminase (SGOT), LDH-total, and LDH-heat stable (19). As you expand this particular model into 12 to 20 variables, misclassification becomes a very significant problem which must be considered when you interpret information received from a mass screening machine using normal limits obtained for one variable at a time.

Multivariate analysis allows a statistical solution to this particular problem, but it is something that is not easily performed within the normal confines of the clinical laboratory. Special studies of normal subjects need to be conducted in order to

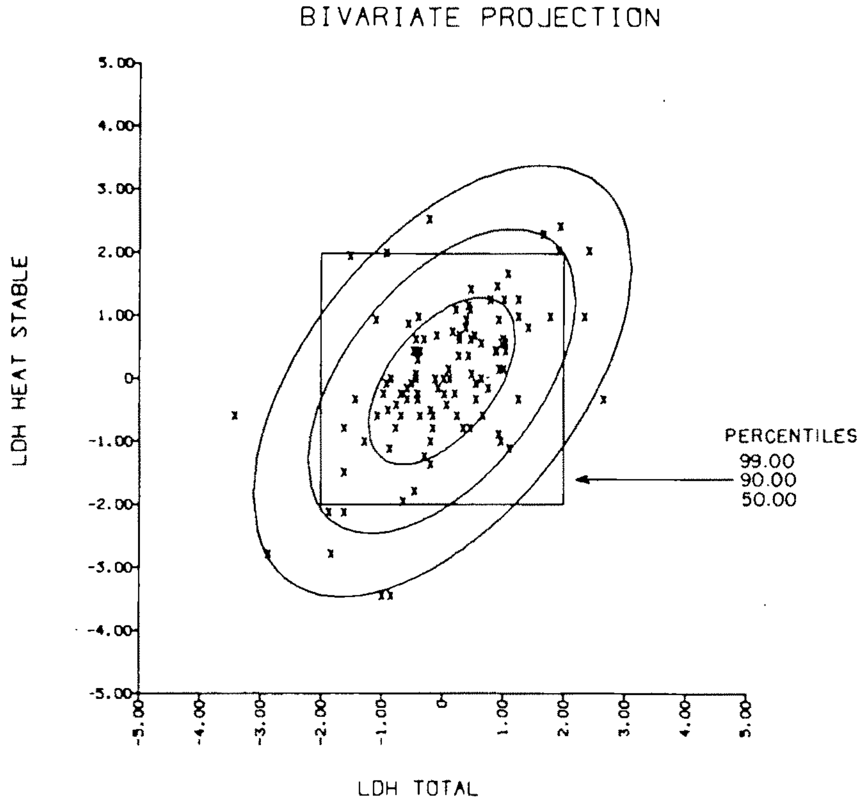

*Figure 1* The normal range for enzymes LDH-total and LDH-heat stable are plotted simultaneously using standard deviate notation. The $x$'s represent the actual patient data pairs. The square shows the classic univariate normal range for the ninetieth percentile. The middle ellipse corresponds to this same ninetieth percentile, yet is the accurate statistical representation of this region. The square misclassified many individuals who would be properly segregated by using multivariate comparisons.

establish the necessary statistical data to develop these types of ellipses. To deliver this advanced analytic program, we will need expansion of the hospital and laboratory computer systems currently in use at many larger institutions (20, 21). Future development in the laboratory will certainly be directed toward this particular area, but will lag behind the utilization of these new mass screening instruments.

## SUMMARY

The clinical laboratory is in a stage of change which has been the direct result of an influx of technology and the current demand for lower costs and increased

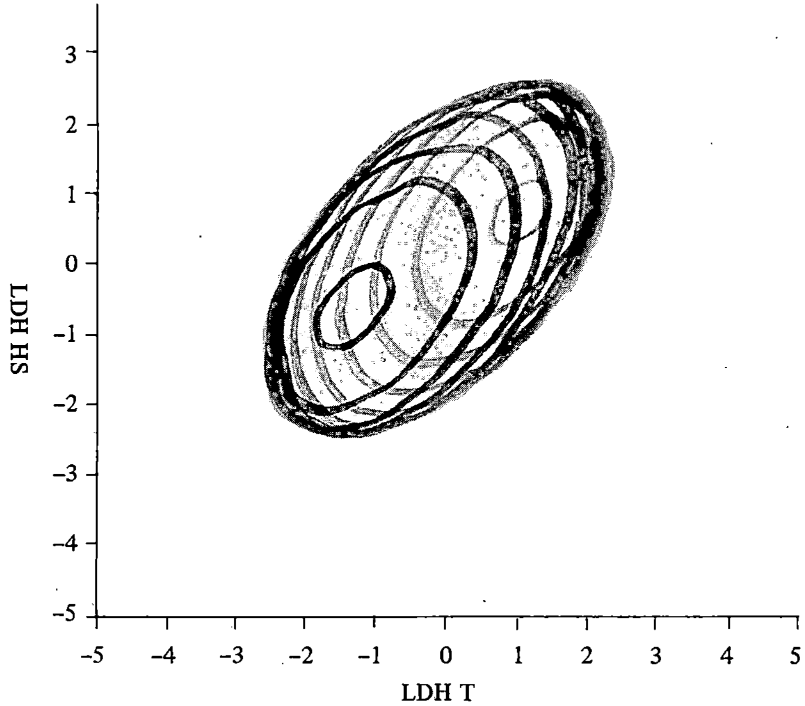

*Figure 2* Multivariate normal can extend to *n* space where planes of normal can be defined. Enzymes LDH-total, LDH-heat stable, and SGOT are shown in 3-D space at the ± 2 standard deviation cutoff. This area of normal corresponds to a football with an axis and shape dependent upon the type of test correlations found.

productivity. There is no significant trend anticipated in the future which would forestall this particular process. The practicing physician can anticipate a fairly marked reduction in the cost of individual tests and, likewise, a marked increase in the amount of available information. There will be a larger clerical function involved in the review of such information along with a series of relevant problems related to the actual size of the new data base. One of the most significant problems related to this increase in numerical reporting will be the statistical problem of the "abnormal-normal." Statistical misclassification must be considered with each abnormal test and a specific approach must be taken to these tests to minimize the inconvenience to the patient. It is recommended that any unsuspected abnormal test(s) that is discovered be repeated as a single test(s) through a reference method. Reordering of the test battery or screen will only offer another possibility for statistical misinterpretation. If the test on isolated evaluation does appear abnormal, then this should call for the routine evaluation of this particular condition. Multivariate statistical analysis could eliminate this probability dilemma, yet is presently experimental and is not in routine use within any clinical setting. We can look forward to the evolution of such a process through the mechanism of a hospital and laboratory communication system which will allow the physician to work with an evaluation process capable of handling these large volumes of numbers (22). Such programs are on the horizon and should be anticipated within the next five years in some of the larger centers that have this new instrumentation.

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# THE FUNCTIONS OF THE MACROPHAGE IN MALIGNANT DISEASE

❖7194

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## MULTIPLICITY OF ROLES

In the past many different names were given to cells which we now realize are all macrophages. This came about because of the different appearances macrophages manifest when carrying out their diverse functions in different locations. Evidence that the immediate precursor of the macrophage is a blood monocyte, derived from radiosensitive cells in the bone marrow, is now overwhelming. When appropriately stimulated, macrophages are capable of active multiplication and mitotic figures are commonly seen in mononuclear phagocytes in granulomas, at sites of inflammation, and in the reticuloendothelial (RE) system and the peritoneal cavity following systemic or local treatment with a number of bacterial products (1). Consequently the number of macrophages in the body can be increased both by division as differentiated macrophages and by stimulating the output of monocytes from the bone marrow.

Macrophages together with polymorphonuclear leukocytes constitute the first line of defense of the body against most infections, and specific immunity requiring B- and T-lymphocytes in general comes into play as a back-up mechanism and for long-term protection. The term "cell-mediated immunity" was originally coined to describe protection provided by macrophages, and its extension to specific immune processes, in which lymphocytes are the effector cells, is of relatively recent origin. The idea that macrophages are involved in the rejection of allogeneic cells injected into the peritoneal cavity was advanced more than 20 years ago (2), and their role in the homograft reaction has been investigated extensively (3–6).

Macrophages contribute at several stages to the genesis of specific immunity leading to antibody production and cell-mediated immunity directed against specific antigens (see below). In addition they act with nonspecific opsonins and recognition factors by exerting an antibacterial action which is not specific in the strict immunological sense but has a high degree of selectivity. The recognition by the RE system of effete red cells and the killing of tumor cells by activated macrophages (see below) may reflect the same general phenomenon by which macrophages, when suitably stimulated, respond to defined cell-surface characteristics.

Macrophages synthesize and release into the extracellular fluid a number of biologically important macromolecules, such as components of complement, transferrin, interferon, and many hydrolytic enzymes (7). Among the latter is lysozyme, which provides powerful protection of the host against many gram-negative organisms. Indeed, lysozyme-sensitive organisms are usually nonpathogenic due to the universal presence of this enzyme in body fluids. Other lytic enzymes released by macrophages, such as collagenase, probably have important physiological roles and contribute to the pathology of inflammation and autoimmune diseases and perhaps invasion of normal tissues by tumors. Some of these lytic substances, e.g. lysozyme, are produced at an approximately constant rate by macrophages and monocytes, whatever their site or stage of stimulation. The synthesis of others, e.g. plasminogen activator, occurs at a higher rate following stimulation, while some enzymes like collagenase are not produced unless the macrophage is activated (7, 8). Finally, macrophages scavenge tissue debris, detoxify certain chemical substances, and participate in the metabolism and disposition of iron, lipids, and proteins.

In view of this wide range of functions, presumption is on the side of the investigator who sets out to determine a role or roles for macrophages in neoplasia. Indeed, it seems likely that such cells will affect at many levels the occurrence, natural history, and response to therapy of tumors. There is an extensive literature (9) on the effect of tumors on the function of the RE system, usually assessed by measuring the rate of clearance of colloids. In some situations the RE system is stimulated while in others it is depressed, and frequently no change is observed between the tumor and normal host. In such studies it is difficult to distinguish between cause and effect, but the available data make it unlikely that a lesion in the RE system is a determining influence in the progress of neoplasia. However, depression of the RE system may facilitate the growth and spread of tumors, not necessarily because of a defect in clearance, but by interfering with the tumoricidal properties of macrophages; it is to this aspect that this review is largely directed.

## ROLE OF MACROPHAGES IN THE GENESIS OF SPECIFIC (LYMPHOCYTE-DIRECTED) IMMUNITY

### *Afferent Level*

The recognition of many antigens by the specific receptors on lymphocytes either requires or is greatly facilitated by the presence of macrophages. Antigen processing by macrophages can result in chemical modification, or lead to their being presented to lymphocytes via a component of the plasma membrane of the macrophage (10, 11). By analogy, processing of antigens by macrophages is likely to be necessary in order for the host to respond to tumor-specific antigens. Thus, neonatal rodents are more susceptible than older animals to the induction of tumors by viruses and chemicals; often tumors can be transplanted into them with many fewer cells than are necessary to transplant tumor into a 2-week-old mouse. This may be due, at least in part, to a defect in the macrophages of the newborn. While these are fully phagocytic, they lack the capacity to process antigen effectively. The immunological immaturity of newborn mice can be corrected by injecting adult macrophages and is therefore not due to a defect of their lymphocytes (12). Similarly, after low doses of X rays, the critical lesion responsible for a reduction in antibody formation is a deficit in macrophages—presumably due to the killing of precursor cells in the bone marrow. It would be of great interest to test whether the vulnerability to tumors of newborn mice, or of adult mice that have been exposed to ionizing radiation, could be corrected in the same way.

### *Central Level*

Macrophages also play an essential role at a later stage in the genesis of immunity when lymphocytes, once they have been stimulated, develop into effector cells. There are several lines of evidence which indicate that the central activity, which may require cooperation of B- and T-cells, and which invariably requires the antigen-stimulated lymphocytes to proliferate, also requires the presence of macrophages or macrophage products. This has been proved in in vitro synthesis of antibody (13), and most investigators also find that transformation of T-lympho cytes by phytohemagglutinin (PHA) and in a mixed lymphocyte culture requires a small number of macrophages (14). Macrophages are also needed for a related reaction, the conversion of a "memory" T-cell into a cell which has the equipment necessary to kill a specific target cell. For many weeks after immunization with allogeneic or syngeneic tumor lymphocytes, T-lymphocytes are found in blood, lymph, and lymphoid organs. These are not directly cytotoxic, but on coming into contact with the specific target cell, they undergo a change, requiring active metabolism, that results in their becoming able to lyse a specific target cell (15). The memory cell can be distinguished from the effector T-cell by its much greater radiosensitivity. The transition from memory to effector T-cell requires the presence of macrophages. If mononuclear cells derived from blood or lymphoid tissue of immunized animals are totally freed from mononuclear cells, they lose the capacity to kill specific target cells in vitro. This occurs because the transformation of memory T-cells to cytotoxic T-cells which takes place on the addition of the target cells does not occur in the absence of macrophages. Addition of macrophages restores cytotoxicity, although the macrophages play no direct part in the killing of the target cells (16). Products from immune lymphocytes can also render macrophages cytotoxic (see below), and two situations must be distinguished: (*a*) When macrophages are necessary for the memory T-cell to transform to a killer cell, and (*b*) when the macrophage itself is rendered cytotoxic by a product released from the memory T-cell.

### *The Macrophage As an Immunologically Specific Effector Cell*

Macrophages obtained by a variety of procedures can exhibit in vitro immunologically specific cytotoxicity i.e. they recognize transplantation antigens (17–19) and tumor-specific antigens (20) in the membranes of the target cells, with a specificity similar to that shown by antibodies or cytotoxic T-cells. The term *cytotoxic* is used to describe all the actions that interfere with normal growth of target cells. This interference may be either rapid lysis or growth inhibition. In all these reactions the primary process occurs during cell-to-cell contact between the macrophage and the target cell, and phagocytosis, when it occurs, follows on the death of the target cell. Frequently the cytotoxicity of mononuclear cells is assessed in short-term experiments in which the target cells are labeled with radioactive chromium, and the release of this label following cell lysis is measured over a period of 4–20 hours. Such tests will not detect slower cytolytic processes or growth inhibition. To recognize all elements of the cytotoxicity, an assay is used in which either the number of target cells remaining in contact with the cytotoxic macrophages after 48 or 72 hr is counted, or their capacity to synthesize DNA is measured using a suitably labeled radioactive precursor. We (21) define lytic macrophages as those which cause release of label within a few hours, whereas growth-inhibiting macrophages are those which stop DNA synthesis, but do not cause the release of isotopically-labeled cell components within 24 hr. When lysis occurs after contact for 2 days or more with specifically cytotoxic macrophages, it is difficult to distinguish whether lysis is a result of prolonged growth inhibition, or is an independent event. The difference between growth inhibition and lysis is not absolute, and macrophages that are principally growth inhibitory to one type of cell may be lytic when directed against another target. The factors that determine which of these processes occurs have not been clearly defined, although the condition of immunization of the macrophage donor appears to play a role (23, 24).

### *Macrophages As Effectors in Cell-Dependent Antibody Cytotoxicity*

Macrophages, monocytes, and lymphocytes that have the "Fe receptor," which binds aggregated IgG or immune complexes via the Fc portion of the Ig molecules, are cytotoxic to target cells coated with IgG antibody and directed to surface antigens of target cells (25–27). In this reaction the effector cell functions in some ways like complement, i.e. the effector cells bind to the target via the Fc portion of the antigen-antibody complex on the target cell, and this interaction is cytotoxic and ranges from cytostasis to lysis as the concentration of antibody is increased (27).

## THE NATURE OF THE SPECIFICALLY CYTOTOXIC MACROPHAGE

### *Arming by Immune Lymphocytes*

Macrophages become specifically cytotoxic on binding to an antigen-specific cytophilic factor, the production of which requires the participation of immune lymphocytes and does not arise in T-cell–deprived mice (15). Whether cytophilic IgG or IgE can arm macrophages in the sense that they become specifically cytotoxic to tumor cells is not yet clear, but they can be armed by a product released when specific antigen is incubated with lymphocytes derived from mice immunized with either an allograft (when the reaction is directed against transplantation antigens), or a syngeneic tumor (when the reaction is directed against a tumor-specific antigen) (28–32). Several experiments (31, 33, 34) indicate that the active material is released from immune T-cells, and arming activity cannot be obtained from lymphocytes of mice deprived of T-cells (55). This activity has been referred to as specific macrophage arming factor (SMAF) and is found in two molecular weight ranges: 40,000–100,000 and greater than 300,000 (31, 34).

SMAF binds to macrophages obtained from normal mice and these then become specifically cytotoxic. The relationship of SMAF to other antigen-specific T-cell products such as antigen-specific migration inhibition factor (MIF) (35), the factors which replace T cells in antibody synthesis and related reactions (36–39), remains to be elucidated. The identification of SMAF has been rendered difficult by the fact that immune lymphocytes can also release a factor which activates macrophages and renders them nonspecifically cytotoxic (see below) (40). It is not known whether a serum factor (41), that is cytophilic for macrophages and isolated from immune serum which moves electrophoretically like albumin, has SMAF activity. Macrophages can also be rendered bactericidal to *Listeria* by a supernatant obtained, like SMAF, from guinea pigs immunized with *Listeria* (42), but the relationship between specific intracellular killing of bacteria and the extracellular killing of tumor cells is not clear, and they may occur by different biochemical mechanisms.

It is possible that high molecular weight SMAF may be a soluble complex of antibody and antigen derived from cell surfaces which renders macrophages cytotoxic by binding the Fc receptor on the plasma membrane of the macrophage (43). Specific target cells would bind to the "armed" macrophage by displacing the antigen in the complex with the antigen present on their target cell membrane. The net effect would be a binding of the target cell via an antigen-antibody complex to the macrophage, as is the case when an antibody-coated target cell binds to a macrophage in cell-dependent antibody cytotoxicity. It has been found in vitro that resistance to challenge with intravenous tumor cells can be transferred passively with the serum from tumor-bearing rats that contain no free antibody but only soluble antigen-antibody complexes (44).

Macrophages can also be armed by incubation with lymphoid cells from hyperimmune mice (20, 30, 32, 45–47). This reaction requires direct contact between the macrophages and the lymphocytes, but occurs, unlike SMAF production, in the absence of antigen. Presumably, lymphocyte products with specificity for the antigen are transferred to the macrophages, but nothing is known of the nature of the substance involved. This experiment was initiated by us following the observation of Mackaness (48) that macrophages which were bactericidal for *Listeria* could be produced by the injection of immune lymphocytes. Similarly, when immune lymphocytes were added to blood leukocytes from nonimmune animals, cytotoxic macrophages were induced (49).

### *Specifically Cytotoxic Macrophages Isolated From the Peritoneal Cavity*

Macrophages derived from the peritoneal cavity (PC) of mice immunized with allogeneic cells are specifically cytotoxic in vitro and in vivo to tumor cells bearing the same surface alloantigens (17–19). If immunization is by the intraperitoneal (i.p.) route, specifically cytotoxic macrophages can only be detected a few days after all of the ascites cells have been rejected; at earlier times the macrophages will be nonspecifically cytotoxic (see below). Macrophages can also kill tumor cells by a reaction directed against tumor-specific surface antigens, and the macrophages from mice immunized with radiation-killed syngeneic tumor cells were found to be specifically cytotoxic to these tumor cells (20, 30). Cytotoxic PC macrophages can also be detected in animals bearing a progressively growing subcutaneous tumor (50). These macrophages kill not only in vitro but also in vivo, as evidenced by the fact that tumor cells injected into the PC are rejected specifically by animals bearing a subcutaneous tumor.

Whether the specifically cytotoxic PC macrophages from immunized or tumor-bearing animals are produced in vivo by one of the arming processes described above is not clear. However, it is apparent that lymphocytes are necessary since no such macrophages are found in animals deprived of T-cells by neonatal thymectomy or by adult thymectomy followed by whole-body radiation (15). Mice treated in this way retain approximately 5% of their normal T-cell complement and have the capacity to make cytotoxic antibodies to alloantigens. This suggests that there were sufficient T-cells for cooperation with B-cells in the induction of the immune response, but that the number of immune T-cells (or effector T-cells) was greatly reduced. If a product from immune T-cells is necessary for macrophages to become specifically cytotoxic in vivo, this would explain the absence in T-deprived animals of cytotoxic macrophages, in spite of the fact that they still make antibodies.

### *Different Types of Specifically Cytotoxic Macrophages*

Cytotoxic macrophages from the PC of immunized mice appear to be of several types, although they display the same specificity to surface allo- or tumor antigens (see Table 1). They can be distinguished on the basis of their response to radiation (23). In terms of survival and capacity to phagocytose, all the macrophages are highly radio resistant, but the capacity to process antigen (22) and the specific cytotoxicity (23) of some macrophages can be ablated by small doses of X rays. The exact conditions needed to obtain the more radioresistant cytotoxic macrophages have still to be elucidated. Reference has already been made to the fact that in the

**Table 1** Classification of cytotoxic macrophages

| Specificity | Mode of action | X-ray dose needed to inactivate |
|---|---|---|
| Directed to histocompatability antigens, and in syngeneic systems to tumor-specific antigens. | Inhibition of DNA synthesis[a] | < 500 R |
| | Rapid lysis | 1000–3000 R |
| Nonspecific; transformed cells are much more susceptible than nontransformed cells. | Inhibition of DNA synthesis[a] | >5000 R |

[a] Some target cells also undergo lysis but for most target cells this is a late event.

same system (e.g a specific immunization with allogeneic cells), the macrophages obtained are sometimes capable of rapidly lysing target cells, whereas at other times only cytostatic activity can be detected, and that the conditions of immunization (in particular the use of live as opposed to radiation-killed immunization cells) are some of the factors that determine whether rapidly lytic macrophages that appear to be also more radioresistant are produced (23, 24). In general, macrophages which are cytostatic and radiosensitive arise whenever an animal is immunized, but the conditions required to produce lytic and radioresistant macrophages are more stringent. The difference between the growth inhibition and lytic macrophages—at least in the systems studied by us—does not seem to be quantitative, i.e. a different intensity of cytotoxic activity, because the lytic cells have different properties such as radioresistance and greater ease of detachment from culture vessels. The latter may lead to the loss of lytic macrophages under certain experimental conditions and their existence can thus be overlooked. There is no doubt that both are macrophages and that the phenomenon is not an artifact due to contamination with lymphocytes. As yet little is known of the biochemical basis for either the cytostatic or the lytic reaction.

## CONTRIBUTION OF SPECIFICALLY CYTOTOXIC MACROPHAGES TO TUMOR IMMUNITY IN VIVO

Cell-mediated immunity is important for the rejection of syngeneic tumor cells by experimental animals following specific immunization with living or killed tumor cells. The nature of the specifically cytotoxic effector cells responsible for causing this reaction in vivo has not been established; however, on the basis of in vitro data there are a variety of candidates, which include lymphocytes with B- and T-cell characteristics, and macrophages. The fact that frequently immunity to challenge has not been attained if the animals had been deprived of T-cells does not necessarily imply that T-cells are the only effector, since T-cells may act cooperatively at various stages in the induction of immunity. There are several types of cells that effect specific killing and the relative importance in vivo of these different effectors varies depending on the anatomical sites. This is brought out clearly by comparing the rejection of inoculated tumor cells in animals that have been immunized with tumor, but are free of tumor, with those in which the tumor under test is actively growing; the latter situation is referred to as concomitant immunity. In general, immunized animals reject the specific tumor cells with approximately equal efficiency whether injected subcutaneously (s.c.), intramuscularly (i.m.), intraperitoneally (i.p.), or intradermally (i.d.). In tumor-bearing animals resistance to s.c.- and i.m.-injected tumor cells is much weaker than for i.v. or i.p. challenges (50–53). This suggests that the presence of the tumor impairs those effector cells which predominate at i.m. and s.c. sites to a greater extent than the effector arms which contribute to the rejection in the peritoneal cavity or the lung.

That immunologically specific cytotoxic macrophages are capable of controlling tumor cells in the lung and the peritoneal cavity is shown by immunotherapeutic experiments in which the injection of specific immune macrophages stops tumors that had been implanted i.p. (50) and i.v. (54). It is questionable whether specifically immune macrophages can be used realistically for immunotherapy, since they do not recirculate and essentially act only at the sites where they are fixed after injection (i.e. the lung following i.v. inoculation, and the peritoneum following i.p. inoculation).

Another approach used to assess the role of macrophages in the tumor-host relationship is to deplete immune animals of macrophages. However, attempts along these lines in our laboratory using antimacrophage serum or silica, which after phagocytosis kills macrophages, have only shown small effects. However, this may be due to the fact that the total fraction of the body's macrophages that can be removed in this way is small. Unfortunately, a mouse devoid of macrophages has so far eluded the experimentalist, and indeed one might argue that such a state is incompatible with survival. A short prolongation of survival of allografts has been achieved by the administration of silica (55).

There are indications that when immunity is transferred by immune lymphoid cells, the adoptively acquired capacity to reject tumor cells is brought about by macrophages that have become armed by the lymphocytes. Indeed, we began to study the role of macrophages in tumor immunity when we failed to find in mice, which were resistant to syngeneic lymphoma cells, lymphocytes which were cytotoxic in vitro. Yet lymphocytes from such animals could transfer the specific immunity to another mouse (20, 56). In the immune mice, we found macrophages which were cytotoxic and could demonstrate arming of macrophages by lymphocytes from immune mice in vitro (20). Similar findings were subsequently observed in other tumor systems, in which the role of the host macrophage in the rejection of tumor cells in animals that had received immune lymphocytes was clearly demonstrated (57).

## NONSPECIFICALLY CYTOTOXIC (OR ACTIVATED) MACROPHAGES

Table 2 lists a number of procedures which in vitro and in vivo render macrophages obtained from the peritoneal cavity cytotoxic to nucleated cells by an interaction that does not involve recognition of specific antigens in the membrane of the target cell. This reaction, like that of the specifically cytotoxic macrophages, is distinct from phagocytosis, which occurs only after the cells have been killed, and follows cell-to-cell contact, though it may involve a diffusible factor released by the macrophages.

Initially we referred (58) to these nonspecifically cytotoxic macrophages as being activated. While this terminology has the advantage of brevity, it has led to confusion, because the term *activation* has been used by others previously to describe changes induced in macrophages that are revealed by morphological, biochemical, and physiological criteria which do not necessarily correlate with cytotoxic potential. While nonspecifically cytotoxic macrophages have many of these properties, there are some procedures that will cause activation according to these physiological criteria but do not render macrophages nonspecifically cytotoxic (59). It is also not known whether treatments which increase the intracellular killing of microorgan-

**Table 2** Procedures that give rise to nonspecifically cytotoxic (activated) macrophages

1. Treat with endotoxin, lipid A, or dsRNA (synthetic or of viral origin) in vitro or in vivo.
2. Exposure in vitro (not tested in vivo) to
    (*a*) peptoglycans from bacterial cell walls;
    (*b*) aggregated IgG (antigen-antibody complexes?);
    (*c*) lymphokines released by antigen from immune lymphocytes.
3. Treat in vivo (but not effective in vitro) with *Corynebacterium parvum* or pyran copolymer.
4. Add, in vitro or in vivo, specific antigen to macrophages from (or in) immunized host (i.e. transformation from armed to activated state).
5. Macrophages from animals with persisting infections of bacteria, protozoa, and nematodes (virus?). Mechanism related to 4 above.

isms in macrophages necessarily render them nonspecifically cytotoxic to mammalian cells.

The first method used to activate macrophages—and in this test this term will be restricted to describe the capacity to kill tumor cells nonspecifically—was exposure to minute quantities of endotoxin, lipid A, or double-stranded RNA, after which the macrophages inhibited the growth of lymphoma and sarcoma cells in vitro in spite of the fact that the agents used were not toxic to such cells (58). It is of particular interest that a low molecular weight peptoglycan isolated from mycobacteria, which is a very potent immune adjuvant, also renders macrophages nonspecifically cytotoxic, and this suggests that these two phenomena may be interrelated (60). Aggregated IgG and some immune complexes also render macrophages cytotoxic (61). Products from immune lymphocytes may also do this (40). In addition to releasing SMAF, immune lymphoid cells when cultured with a specific antigen produce a soluble factor which renders macrophages nonspecifically cytotoxic. The relation of this lymphokine to MIF and other active substances found in such preparations remains to be resolved.

Macrophages from suitably immunized mice become nonspecifically cytotoxic after incubation with the relevant antigens (62). If the mice have been immunized with allogeneic cells, the specific cytotoxic macrophages kill nonspecifically, following incubation with the antigens involved. The change from "arming" to "activation" is shown diagrammatically in Figure 1. Alternatively, macrophages from mice immunized with a bacille Calmette-Guérin (BCG) of low virulence (62), or with sheep red blood cells (63), are not cytotoxic but become so after contact with BCG [or purified protein derivative of tuberculin (PPD)], or with sheep red blood cells. This process may explain why nonspecifically cytotoxic macrophages can be found in the peritoneal cavity of mice carrying persistent infections such as *Toxoplasma gondii, Besnoitia, Listeria* (64), *Pasteurella* (62), or nematodes (65). Strains of BCG which give rise to persistent infections in mice cause the appearance of "activated" macrophages (66, 67), whereas other forms of BCG (like that produced by Glaxo

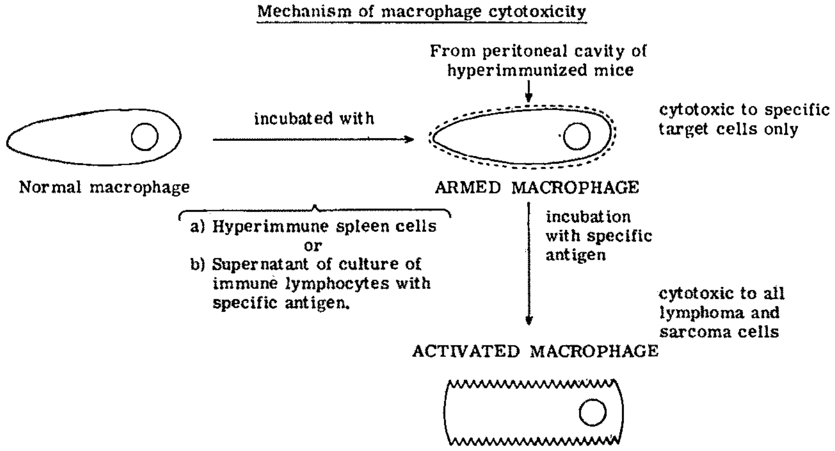
Mechanism of macrophage cytotoxicity
From peritoneal cavity of hyperimmunized mice
incubated with
cytotoxic to specific target cells only
Normal macrophage
ARMED MACROPHAGE
a) Hyperimmune spleen cells
or
b) Supernatant of culture of immune lymphocytes with specific antigen.
incubation with specific antigen
cytotoxic to all lymphoma and sarcoma cells
ACTIVATED MACROPHAGE

*Figure 1* Mechanisms of macrophage cytotoxicity: transition from the "armed" to the "activated" state.

Laboratories Ltd.), which are rapidly killed in mice, only produce cytotoxic macrophages after they have been incubated with the specific antigen, such as PPD (62).

### *The Increased Susceptibility of Tumor Cells to Activated Macrophages*

Hibbs & Remington (68, 69) made the remarkable observation, subsequently confirmed (40, 70), that the cytotoxic action of activated macrophages, while nonspecific in an immunological sense, still shows selectivity in that transformed cells (i.e. malignant cells) are much more susceptible than are their normal counterparts. This difference in susceptibility is not caused by differences in the rate of cell division, although there have been claims that malignant cells which divide rapidly are more sensitive to activated macrophages (71, 72). The biochemical basis of this discrimination is unknown, but it is tempting to speculate that it is a development of the primitive protection mechanisms of invertebrates exercised by phagocytic cells. It is not clear whether the discrimination is absolute, and there have been claims that activated macrophages stop DNA synthesis of PHA-transformed lymphocytes (73), kill cells infected with para influenza virus (74), and lyse red cells (75). There are experiments which suggest that the difference may be one of degree, and that rapidly dividing normal cells may be growth inhibited but not lysed by activated macrophages (71, 72).

### *Mechanism of Cytotoxic Action*

While some malignant target cells lyse rapidly after exposure to activated macrophages, in general the first effect is cytostasis, followed a day or so later by cell lysis. For this reason the cytotoxicity of activated macrophages would be missed in short-term tests, such as the release of radioactive chromium from cells within 6 hr, a procedure frequently used to measure the cytotoxicity of T-cells. Several investigators (58, 64, 67, 71) find that direct contact between the activated macrophage and the target cell is necessary for cytotoxicity, and an exposure of 6 hr or more is required for irreversible damage to become manifest after endotoxin treatment (58). In other studies (76–80), different supernatants derived from activated macrophages having a wide range of properties and molecular weights were found to be cytotoxic. Their activity disappeared rapidly on dilution, and whether one or more of the substances is responsible for the potent effects seen with intact macrophages remains to be established. Exocytosis of lysosomes has been proposed as the lethal step (81); however, a factor (85) extracted from endotoxin-activated macrophage which is lytic for transformed cells but not for normal cells, is unstable, and is antagonized by serum proteins seems a promising candidate as the active principle. An attractive hypothesis is that the cytotoxic action is mediated by a hydrolytic enzyme (e.g. protease or lipase) released by macrophages, but that its action is antagonized by components in the serum, either by competition or direct inhibition. Consequently, slight changes in the experimental design may determine whether a cell-free supernatant is active or not. Reference has already been made to the fact that macrophages release many biologically active molecules, and that the rate of release of some of these materials, and in particular enzymes capable of hydrolyzing macromolecules, is greatly increased by treatment which activates macrophages.

### *Activation In Vivo*

As already mentioned, macrophages taken from animals with persistent infections, or animals that receive an injection of antigen to which they are sensitive, are activated. Intraperitoneal injections of endotoxin or double-stranded RNA causes macrophage activation in vivo, although such cells can usually only be recovered a few days after inoculation because their first and transient effect is to cause the peritoneal fluid to become acellular (58). Two potent stimulants of the reticuloendothelial system, *Corynebacterium parvum* (82) and a synthetic pyran copolymer (83), do not activate in vitro, but cause activated macrophages to arise in vivo within a few days of their injection, possibly as a result of the release, following metabolic digestion, of an activating substance.

### *Nonspecific Cytotoxic Monocytes*

It is difficult to show experimentally that monocytes can be activated by the procedure described above, and if activated, whether they turn into cytotoxic macrophages or are cytotoxic as activated monocytes. The investigators studying cell-mediated immunity to human tumors frequently find that mononuclear cells from the blood are nonspecifically cytotoxic to tumor cells, and this has caused a great deal of confusion concerning the nature of the host reaction to tumors in man. In many instances preparations of mononuclear cells are described as being lymphocytes in spite of the fact that they contain many monocytes. Indeed, the widely employed separation using Ficoll trisil gradients concentrates monocytes, and as many as 30% of so-called lymphocytes may in fact be monocytes. The claim has also been made that many animal tumors contain a common fetal surface antigen because leukocytes from multiparous rodents kill tumor cells in vitro. In this situation monocytes were shown (84) to be responsible for the killing, and it is therefore possible that the test does not measure a host reaction to a widely cross-reacting fetal antigen, but instead records the antitumor action of activated monocytes.

The nonspecific cytotoxicity of immune spleen cells following exposure to antigen or endotoxin has been shown to be due to monocytes and macrophages (91).

### *Activated Macrophages in Carcinogenesis*

We have proposed (21) that activated macrophages may exert a surveillance role in the body against spontaneously arising malignant cells. There are several lines of evidence which suggest that malignant transformations occur much more frequently than do tumors, and that the body eliminates the vast majority of malignantly transformed cells before they can give rise to tumor. The hypothesis was advanced that the in vivo control of malignantly transformed cells occurs via lymphocyte-mediated immunity and requires the participation of T-cells. This concept has run into severe problems because there are several clear and decisive animal experiments which show that mice that have been deprived of T-cells and are capable of accepting foreign grafts, are not more susceptible to spontaneous tumors or to tumors induced by chemical carcinogens (86). The only carcinogenic process to which they are more vulnerable is the induction of tumors by viruses, but in this case it is the failure of the immune system to control the virus that is responsible for the increased tumor incidence. In man also, the hypothesis that the increased incidence of some malignancies in patients who have received kidney transplants is due to a loss of T-cells has been challenged. Rather than abandon the concept that there are in vivo mechanisms by which transformed cells are eradicated, it is attractive to postulate a surveillance mechanism, which is not based on the conventional immune system, but relies instead on activated macrophages. A decisive test of this concept is difficult because of the impossibility of depriving animals of all their macrophages.

A more direct link between macrophage activation and the incidence of cancer is indicated by the findings of Lurie (87) in rabbits. In different strains the natural resistance to tuberculosis was parallel to the incidence, at old age, of uterine cancer, which was least in the most resistant strains. Resistance to tuberculosis was related directly to the efficiency with which tubercle bacilli ingested by mononuclear phagocytes were killed, and it is tempting to associate resistance to cancer with the same cells. This experiment does not of course indicate that the tumor resistance was conferred by activated macrophages. Evidence to support this view comes from the laboratory of Remington (88, 89), in which it was found that mice that were chronically infected with intracellular protozoa (e.g. toxoplasma) were more resistant to induction of tumors by viruses and to tumors resulting from the injection of syngeneic tumor cells. Salmonella infections produce a similar effect. The infected mice (89) contain macrophages which proved cytotoxic to the tumor cells injected by in vitro tests.

### *Activated Macrophages in "Nonspecific" Immunotherapy*

It is an old observation that some well-established tumors in man and experimental animals undergo complete regressions following injection of endotoxin, and more recently, double-stranded RNA has been found to work similarly (90). The mechanisms involved are complex and include damage to the vasculature, but there are indications that activation of macrophages by these agents contributes to this effect (90). There is also evidence in both man and experimental animals that some tumor cells are destroyed in an immunologically nonspecific way at sites of inflammation (92). Thus, more than a million syngeneic sarcoma cells are rejected when PPD is given i.p. to mice that have been immunized previously with BCG (93). Under such conditions activated macrophages are formed, and it seems likely that these contribute to the antitumor action which is seen with some tumors when inflammation is induced in them or surrounds them.

It is well known that following inoculation of certain bacteria and products derived from them, the RE system is stimulated, as revealed by intense hypertrophy of spleen and liver. Animals treated in this way are subsequently more resistant to challenge with microorganisms, as well as some tumor cells. Among the most effective agents for achieving this effect are living BCG organisms and killed *Corynebacterium parvum* organisms. While under some conditions activated macrophages can be produced by the administration of these agents (see above), the increased resistance to challenge is unlikely to be due to activation since it persists long after activated macrophages can be obtained from the animals (92). It is more likely that the resistance is conferred by the increase in the total number of macrophages in the body of the animal. However, the systemic administration of BCG can also slow the growth of some established tumors, and here it seems likely that activated macrophages may contribute (92), although increased clearance of circulating tumor antigen may also play a part (94).

Some tumors, notably those growing in the skin, can be induced to regress when they are injected directly with BCG or *Corynebacterium parvum.* It is not yet clear whether this is due to bringing activated macrophages into the tumor or to other factors associated with the intense local lesion that is produced by the s.c. inoculation of BCG. It is clear (92) that multiple mechanisms are involved in the antitumor action of BCG, and the relative importance of these is likely to vary for different tumors and under different conditions of administration. However, in some situations it would appear that activation of macrophages by BCG via the process of arming to activation, illustrated in Figure 1, is important.

## THE ROLE OF MACROPHAGES WITHIN TUMORS

While the presence of macrophages at the edge of and within neoplasms is well recognized, the number of these is often greatly underestimated because many may not be recognized readily in histological sections. Some experimental tumors were shown (95) to contain large numbers of macrophages by disaggregating the tumor and identifying macrophages in the suspension by a variety of physiological criteria, which included the capacity to spread on glass in the presence of trypsin, the uptake of several opsonized sheep red cells, and susceptibility to a highly specific antimacrophage serum. In this way macrophages can be clearly distinguished from tumor cells, from other inflammatory cells, and from stromal cells (96). These tests revealed that the macrophage content of different rat sarcomas ranges from 3–58% (97). The macrophages were derived from the bone marrow of the tumor bearers, and there is no evidence that they multiplied within the tumors. They are not malignant.

The capacity of the experimental tumors to metastasize spontaneously to lung and lymph nodes was inversely related to their macrophage content; thus 90% of animals with tumors containing 50% or more of macrophages could be cured by surgical removal of their tumors, whereas under comparable conditions 90% of animals with sarcomas having less than 10% macrophages died from distant metastasis following excision of the tumor graft (97). The macrophages in the tumor were shown to be of host origin and to enter the tumor presumably as monocytes, as part of the immune response to tumor antigens. In rats deprived of T-cells but having normally functioning marrow, the macrophage content of tumors was greatly reduced (97) and the incidence of metastasis was increased. In a preliminary survey (98) the macrophage content was measured in 44 human tumors using the procedures developed by Evans; they ranged from 0–30%. While the follow-up has not been sufficiently long in the patients from whom the tumors have been removed, in order to correlate the macrophage content of the malignant tumors with prognosis, there are indications that tumors which metastasize contain fewer macrophages.

In rats implanted with different syngeneic sarcomas there was a close correlation (99) between the macrophage content of the tumors and the capacity of the rat to mount an inflammatory response to unrelated antigens. Rats previously immunized with BCG in sheep red blood cells lost their ability to give a delayed hypersensitivity (DHS) reaction on injection of the relevant antigen. The impairment of the DHS reaction can be related to the sequestration within sarcoma of host macrophages for four reasons: (*a*) the macrophage content of the tumor is proportional to DHS defect; (*b*) the DHS returns to normal after excision of the tumor; (*c*) lymphocytes from DHS-negative tumor bearers transfer immunity; and (*d*) addition of normal macrophages to the eliciting Ag restores DHS reaction in tumor bearers.

There is no decisive data which bears on the role played by the macrophages in the tumor with respect to its growth and dissemination. It is possible that they are irrelevant bystanders brought in by lymphokines released in an immune process effected entirely by lymphoid cells which interact with tumor cells. Conflicting results have been obtained on the in vitro cytotoxicity of macrophages separated from tumors (100–102). In some experiments these macrophages were specifically cytotoxic to the malignant cells of the tumor from which they were derived, in other cases they were nonspecifically cytotoxic, and in yet other experiments they were not cytotoxic at all. This complexity may be related to the procedure used to isolate the macrophages. One might anticipate that a change from specific to nonspecific cytotoxicity may occur during isolation as a result of contact with tumor. The puzzle remains as to why many tumors grow relentlessly in vivo while they contain macrophages. This could mean that macrophage cytotoxicity is an in vitro artifact, or that the macrophages exert some restraint but are overwhelmed. The impressive correlation with metastasizing potential (97) makes it probable that they exert some controlling influence.

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# CIRCADIAN RHYTHMS AND EPISODIC HORMONE SECRETION IN MAN[1]

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## INTRODUCTION

The temporal ordering of physiological and pathological processes has always been of considerable interest to the biological scientist as well as to the clinician. However, the historical dominance of the concept of constancy of the "milieu interior" and biological homeostasis as formulated by Claude Bernard in France and Walter B. Cannon in the United States has directed most past research efforts in the direction of studying stimulus-response and steady-state mechanisms. During the past 25 years, however, the field of biological rhythms has made great strides and has emphasized that all single- and multicell organisms and a wide variety of biological functions within these organisms demonstrate endogenous oscillatory behavior. The disciplines of endocrinology and neuroendocrinology have been at the forefront of these issues because of the critical interface relationsips of the central and peripheral nervous system, endocrine secretory systems, and the blood circulating communicative pathway. During the past ten years there has been a major conceptual change emphasizing the rhythmic oscillatory and episodic temporal ordering of neuroendocrine events in relation to the 24-hr sleep-waking rhythm. The cross-fertilization of the disciplines of biological rhythms, sleep physiology, and neuroendocrinology has led to a series of important observations which have challenged 24-hr homeostatic and steady-state concepts. The use of the techniques of frequent serial blood sampling, sophisticated microchemical assay techniques, and polygraphic sleep stage analysis have allowed studies to be carried out in man correlating large numbers of sequential multiple hormone measurements with sleep-waking functional states. The results of these studies have demonstrated that hormonal control is related to a temporally "programmed" series of events, endogenous circadian (circa=approximately; dian=day) rhythmic processes, and central nervous system complex "state" (including behavioral and affective) relationships. These findings have begun to be applied to certain clinical problems in man, although it is probable that all the endocrine functions will be shown to have rhythmic properties. In this review, I emphasize both the circadian and shorter term episodic patterns of those hormone systems that have been most extensively studied. These include ACTH-cortisol; growth hormone (GH); prolactin; and the gonadotrophins, lutenizing hormone (LH), and follicle stimulating hormone (FSH).

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## THE CIRCADIAN ACTH-CORTISOL SECRETORY PATTERN

The first description of a 24-hr pattern of hormone concentration was that of G. Pincus, who reported a diurnal rhythm of 17-ketosteroids in the urine of normal young men (1). It was subsequently shown that there was a correlated 24-hr concentration pattern of plasma 17-hydroxycorticoids (2–4). Although these early studies were carried out with infrequent sampling (every 3–4 hr), nevertheless it was recognized that the peak plasma values were between 6 and 8 AM (10–15 $\mu g$/100 ml) and peak urine values between 9 and 11 AM; the nadir for both plasma ($<5$ $\mu g$/100 ml) and urine occurred between midnight and 2 AM. In addition, the metabolic clearance rate of cortisol was reported to be higher in the morning than at night, utilizing a 6-hr integrated sampling technique (5). If the sleep-waking cycle in man was inverted, the circadian plasma cortisol circadian curve was also reversed. This inversion did not occur rapidly but took 1–2 weeks (6–8). In addition, when subjects were deprived of sleep for 1 or 2 nights, the sharp rise of cortisol in the early morning hours was not prevented (9).

In order to study the relationship between sleep stage patterns and the plasma cortisol concentrations an indwelling venous catheter technique was devised, to obtain plasma samples at frequent intervals with a minimum of disturbance to the sleeping subject (10). With sampling done at 30-min intervals during the sleeping period, it was found that the rise in plasma 17-OHCS which occurred during the latter half of the sleep period was characterized by a series of episodic peak elevations, indicating that cortisol was being secreted in a series of episodic secretory bursts. This finding was confirmed using $^{14}$C-labeled cortisol and was extended to 24-hr studies (11). A specific one-to-one relationship between the nocturnal episodes of cortisol secretion and the non-REM–REM sleep cycle, however, was not supported by the previous findings in relation to sleep deprivation (9), nor by the dissociation between sleep stage and plasma cortisol patterns when the sleep-waking cycle was inverted (8). In detailed studies of the 24-hr pattern of cortisol secretion it was found that the cortisol secretory pattern was an episodic one throughout the 24-hr day with a greater clustering of the episodes during the latter half of the sleep period and the subsequent morning waking time (12, 13) (Figure 1). Further confirmation of an episodic pattern was found when adrenal vein blood was sampled at 15-min intervals in man and spontaneous pulses of cortisol secretion were measured (14).

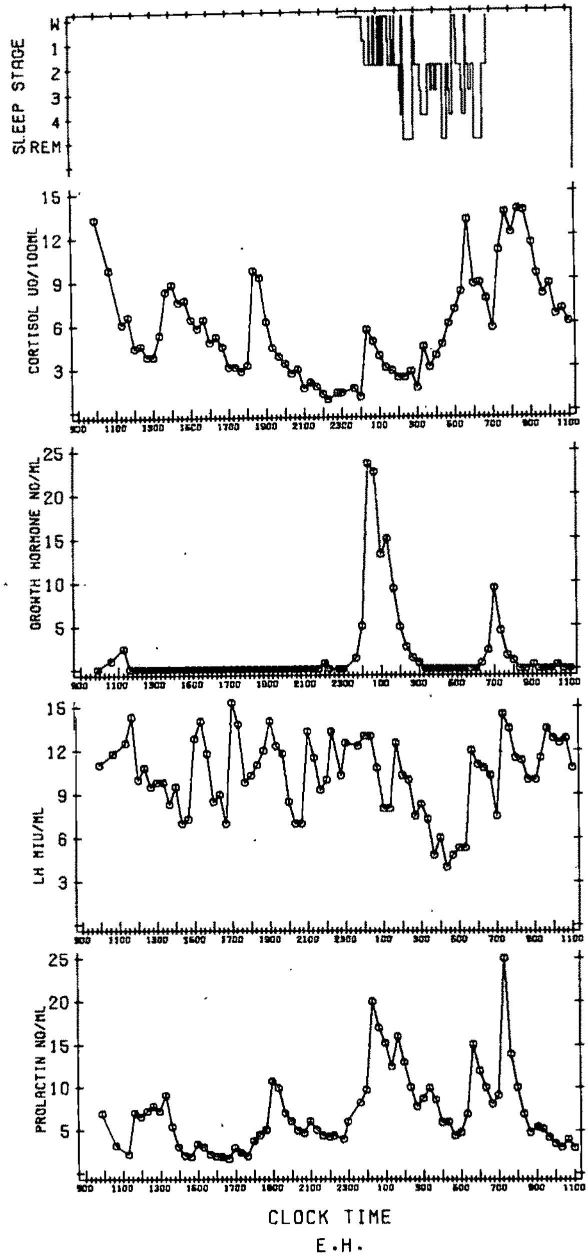

*Figure 1* Twenty-four-hour pattern of plasma hormone secretion in a 21-year-old normal woman for cortisol, growth hormone, prolactin, and luteinizing hormone. Study was done during early follicular phase of the menstrual cycle. Samples were obtained with an indwelling venous catheter and sampling done every 20 min. Sleep stages are defined by polygraphic recording.

Utilizing a 20-min sampling technique (72 samples/24-hr period), it was found that only about 25% of the 24 hr was spent in active secretion with 9 to 11 discrete episodes (12). There were four unequal temporal phases defined, with approximately 40% of the total daily secretion taking place during a 4-hr period in the early morning. During the 4-hr period in proximity to the usual nocturnal sleep onset time (e.g. 10 PM to 2 AM), the cortisol concentration was usually extremely low (frequently zero), and less than 5% of the daily cortisol output occurs during that time period. Cortisol concentrations, obtained at 30-min intervals over 24 hr in normal children, were analyzed by a "pharmacodynamic" mathematical model, assuming sinusoidal circadian and "switch" functions, confirming the episodic nature of cortisol secretion. They emphasize that the episodic and circadian functions account for 89% of the variability, whereas the 24-hr cosine function only accounts for 35% (15).

It is important to emphasize that in normal man a "basal level" or "steady state" of cortisol is not present for any extended time period of the day, and there is considerable variability in the lag time and concentration values between secretory episodes. These findings indicate that a closely controlled homeostatic feedback system is not the mechanism for the normal control of the 24-hr plasma cortisol secretory pattern, but rather suggests that the sequence of episodic secretory events is "programmed" as part of a stable repetitive daily pattern in concert with the sleep-waking cycle and the general program of biological rhythms (16, 17).

The 24-hr episodic pattern of cortisol has been correlated with plasma ACTH measurements in man. These studies have indicated that appropriate temporal correlations are present for the major secretory episodes (13, 18, 19). when a 5-min sampling frequency was used, it was found that several ACTH episodes of short duration in close temporal juxtaposition may produce only one cortisol secretion episode with a smoothly rising concentration.

Several studies have suggested that a correlation may exist between the episodic secretion of aldosterone and cortisol when the frequent intermittent plasma sampling technique was used (20–22). When a 30-min integrated concentration sampling technique was used, a weak but significant correlation was also found (23) with higher episodes of secretion during sleep. Additional evidence for the partial control of aldosterone secretion by ACTH during the 24-hr period comes from the finding that in patients with primary aldosteronism, the 24-hr aldosterone rhythm was temporally correlated with cortisol. Dexamethasone suppression eliminated both the aldosterone and cortisol rhythms (24).

The stability of the circadian episodic pattern of cortisol secretion is demonstrated by several recent studies. In a group of normal subjects who were placed on a 3-hr sleep-waking schedule for ten days, there was no significant difference in the average 24-hr cortisol output, number of secretory episodes, and total secretory time, and all demonstrated a clear circadian rhythm of plasma cortisol compared with the baseline pattern (25). However, a 3-hr cycle of secretory episodes was entrained to the 3-hr sleep wake cycle. A study of seven totally blind subjects revealed that five had a definite circadian pattern of episodic secretion, whereas two had atypical patterns without a clear 24-hr pattern (26). The results of a study of the 24-hr cortisol, growth hormone, and sleep stage patterns during the four seasons of the year in a group of Norwegian air force pilots in Tromso, Norway (above the arctic circle) demonstrated no differences in the circadian pattern of cortisol, although there was a small increase in the mean daily plasma concentration and amount secreted during the autumn–winter compared with the spring–summer seasons (27). A comparison of the 24-hr cortisol and GH pattern was made between two different behavioral schedules of healthy medical students; an "active" day with full, normal activities (classes, meals, driving a car, etc) compared with a "basal" day of strict bed rest (lights on) without reading, conversing, or other external stimulation (28). An episodic cortisol secretory pattern was clearly present for all subjects during both the active and basal 24-hr periods and all had the typical circadian clustering of episodes during both behavioral conditions. However, higher peak concentrations were found during the noon to 8 PM waking portion of the 24-hr day on the active as compared to the basal day. In a recent psychoendocrine study utilizing the frequent (20 min) 24-hr sampling technique, Czeisler (29) compared four patients on the day before major elective cardiac surgery with normal control subjects. The only difference found between the two groups was that a major pulse of cortisol was secreted between 9 and 11 PM for each patient in the presurgery group, clearly caused by the preoperative preparation (body shaving, wash, and enema). The rest of the 24-hr period, including the nocturnal episodic pattern, was the same in the two groups.

These results, taken together, indicate that most of the episodes of cortisol secretion are endogenous and have a "programmed" circadian pattern. Indeed, the interepisode intervals and, therefore, clustering of the episodes appear to be the major determinant of the circadian distribution. The 24-hr pattern is quite resistant to major shifts in the sleep-waking cycle, on a short-term basis, but when the sleep-waking cycle is persistently phase shifted, the 24-hr cortisol pattern will entrain to it. Differences in waking behavior, particularly those associated with strong emotional anxiety, will produce selective but transient episodes of cortisol secretion.

There have been a number of studies applying these new concepts to clinical problems. Abnormalities of the 24-hr plasma cortisol concentration have been found in older patients with psychotic depression (30). Significantly more cortisol was secreted during both the daytime waking and nocturnal hours of sleep with both an increase in the number of secretory episodes and in the time of secretion. After treatment and clinical improvement, the patients' secretory patterns approached normality. The authors suggest that these findings support the concept of a dysfunction of CNS monoaminergic neurotransmitter systems in this psychiatric disease.

Several patients with Cushing's syndrome have been studied and found to have a markedly exaggerated episodic 24-hr secretory pattern (28, 31–34). Although many individual concentrations are not outside the normal range, the pattern is quite abnormal with values rarely falling below 10 $\mu g/100$ ml. This is especially striking in the late evening and early sleep period when normal values are very low and often zero. In a recent report, a statistical analysis was applied to the 24-hr cortisol pattern in Cushing's syndrome, and it was concluded that a 24-hr rhythm was present, although the amplitude of the rhythm was significantly reduced (35). However, in several patients with a proven adrenal cortical adenoma, the range of values throughout the 24-hr day was remarkably narrow (e.g. 8–14 μg/100 ml) suggesting that a 24-hr serial sampling procedure might aid in differentiating bilateral adrenal hyperplasia from adrenal tumors.

In one patient with Addison's disease, 24-hr sampling (30-min intervals) of plasma ACTH and 11-OHCS demonstrated that a circadian episodic pattern of ACTH was clearly present in spite of the absence of a 24-hr plasma cortisol concentration variation (36).

A study of four patients with hyperthyroidism demonstrated that in association with a shortened half-life, there was an increase in both the number of cortisol secretory episodes and the time spent in secretory activity for the 24-hr day (37). The normal decrease of the plasma cortisol concentration to very low values at sleep onset was clearly present, indicating that a circadian pattern was preserved in spite of the major metabolic peripheral and central nervous effects of the presence of excess thyroid hormone.

## TWENTY-FOUR-HOUR PATTERN OF GROWTH HORMONE (GH) SECRETION

It is now well established that GH is secreted in substantial amounts during the first 2 hr of sleep at night when the subject has a normal sequence of nocturnal sleep stages (38–41). Following sleep onset at night and with the characteristic progression of sleep stage patterns (Stage 1⟶Stage 2⟶Stage 3–4), the rapidly rising concentration of GH can be detected within the first 30–45 min, usually reaching a peak between 1–2 hr (Figure 1). Following this major episode of secretion, the concentration rapidly falls to extremely low concentrations, usually zero by the third hour of the sleep period. In some cases a second or rarely a third smaller secretory episode may occur during the remaining hours of the nocturnal sleep period. In repeated studies, this sleep-induced release has been found to be the most consistent time of GH secretion for the 24 hr. In the study of the Norwegian pilots described earlier (27), in only 2 of the 28 24-hr GH measurements did a daytime GH peak exceed the peak at sleep onset at night. During the waking portion of the 24-hr period, infrequent short episodes of secretion occur, but no consistent temporal pattern has been found. For most of the 24 hr in normal subjects, the plasma GH is either undetectable or at very low concentration (<3 ng/ml). If sleep onset is delayed or prevented for many hours, the expected GH release will also be delayed and will then occur in relation to the delayed sustained sleep period (42). This sleep-related release has been correlated to the Stage 3–4 sleep in several studies (41–43). Stage 3–4 sleep characteristically occurs within the first 3 hr after sleep onset at night. In a study carried out by our group (44), we found that the release of GH coincided with the development of the EEG pattern characterized by slow synchronous activity ("slow wave" sleep) in the period after sleep onset at night. However, slow wave (stage 3–4) sleep is not always correlated with GH release since it has been found that especially during the latter portion of the night, Stage 3–4 occurred without an associative GH secretory episode (41). In a study of the ontogeny of the 24-hr pattern it was reported that in four prepubertal males (ages 8–15) GH was secreted only during sleep and not during the waking portion of the day (45). In pubertal adolescents (ages 9–20), however, there were frequent secretory episodes (1–4) during waking, as well as several episodes (1–4) in sleep. There was a 7.5-fold increase in estimated total amount secreted in pubertal children, compared with the prepubertal group. In young adults (ages 23–42) GH was secreted during both waking and sleep, although less so than in the pubertal group, and in an older adult group (ages 47–62) there was a great reduction in GH secretory episodes, with three of the five normal subjects showing no GH at any time during a 24-hr study period.

In several more recent reports (46–48), using the technique of integrated sampling (continuous pump withdrawal of blood with sampling at 30-min intervals), it is reported that prepubertal children do secrete GH during the waking state as well as during sleep, but the amounts are less in prepuberty compared to puberty; adult subjects were also found to secrete less hormone than pubertal children. In infants it has been shown that GH has a high plasma concentration range throughout the 24-hr day, although a sleep-waking relationship has not been well documented (49–51).

The relation between daytime napping and GH secretion (52, 53) was clearly shown in a study recently carried out by our group (54, 55) in which 15 of 18 secretory episodes during the daytime for the six subjects on a "basal" day (see previous section) were clearly related to a polygraphically defined daytime nap. In the "activity" day group without naps, there were 11 daytime secretory episodes.

It is of course well known that other stimuli will induce a GH secretory episode during the day in man (56). These include arginine infusion, insulin hypoglycemia, venipuncture, nonspecific stress, and L-Dopa (57–61). It has been considered that there is a relationship of GH secretion to meals (postprandial rise); recent work has challenged that concept with evidence that there was no reproducible GH secretion temporally related to meals (62). Although an earlier study did not report an increase in GH secretion during nocturnal sleep after heavy daytime exercise (63), a more recent study did show an increased amount and earlier rise of the sleep-related GH secretion, but without a concomitant increase in stages 3–4 sleep in 10 of 12 subjects studied after heavy daytime exercise in nonathletes (64).

The sleep onset GH secretion is not associated with changes in plasma glucose (65) or insulin (66), nor can it be suppressed by an intravenous infusion of glucose or a 5-day high-carbohydrate diet (52). Although it has been reported that medroxyprogesterone acetate in pharmacological doses will diminish the sleep release, it was recently reported that there was no change in sleep-related GH secretion in women comparing mid-and early menstrual cycle sleep periods (61). All of these findings suggest that the sleep-related release of GH utilizes a different pathway of CNS control, related in some way to neural mechanisms underlying the non-REM sleep process.

The recent finding that the polypeptide somatostatin will inhibit the sleep-related GH secretion (67), as well as the arginine- and L-Dopa-initiated release (68), suggests that the strong inhibitory substance acts at a point distal to the sleep-related stimulatory site.

We studied the GH secretion during sleep in a group of seven blind subjects, and found that six had clear sleep-related release of GH (26). The two subjects with low or absent sleep GH release had the most abnormal sleep pattern with markedly reduced Stage 3–4 sleep. The report of Krieger & Glick (69) indicated that GH was absent during sleep in their group of blind subjects, but their sleep patterns were quite abnormal and therefore may explain in part the different results. The importance of relating GH secretion during sleep at night or during daytime naps to polygraphically defined sleep stages is strongly emphasized.

The application of these findings to clinical problems has been increasing. In each of five patients with acromegaly, the 24-hr pattern of GH differed from normal (70). The GH concentration never reached undetectable levels during the 24-hr sampling period (every 20-min sampling). In addition, rapid changes in concentration were found, indicating frequent distinct episodic secretions. Therefore, although GH concentrations in several patients were found to be within the normal range, the 24-hr pattern was grossly abnormal and resulted in markedly increased GH production over a 24-hr period. In these patients the 24-hr curve appeared to have a circadian pattern, with high values at approximately 4 AM and low values at 4 PM, although a clear sleep-related release was seen in only one patient.

The nocturnal sleep pattern of GH secretion has been studied (every 30-min sampling) in a group of ten children with cerebral dwarfism, a syndrome consisting of mental retardation, growth retardation, and other evidence of neurologic abnormalities (71). It was found that five children had no significant increase in GH during sleep. Although polygraphic measurements were made, no report was given about the relation of GH concentration changes to sleep stage pattern. It has been shown that GH abnormalities may be present in certain children with growth retardation, such as those associated with emotional deprivation (72), kwashiorkor (73), and isolated GH deficiency (74). The 24-hr GH secretory pattern, particularly related to polygraphically defined sleep stages, has not been reported in these groups in comparison with the normal pattern and is certainly an area of investigation that should lead to clinically applicable results. In a retrospective study by Wolff & Money (75), of patients with "reversible somatotropin deficiency," also known as psychosocial dwarfism, a good correlation was reported between the estimated quality of sleep and growth rate. However, neither GH nor sleep was measured. The actual measurement of GH during sleep in a group of children with short stature did not reveal a GH deficiency nor an abnormality in sleep patterns (76). However, in children with hypopituitarism and short stature, GH release in sleep was either absent or markedly deficient (77, 78). Comparison with either stimulatory tests (arginine infusion and insulin-induced hypoglycemia) indicates that the measurement of GH release in sleep in association with polygraphic monitoring is a valuable diagnostic test.

It has been reported that both Stage 3–4 sleep and the sleep-related GH release are diminished to absent in several patients with Cushing's disease, Cushing's syndrome with an adrenal or a pituitary adenoma, hypothalamic tumor, and in a patient with Nelson's syndrome (79). However, patients receiving chronic corticosteroid medication had a less significant depression of the nocturnal increment in GH. These studies raise the interesting possibility that in Cushing's disease there is altered CNS function independent of the associated hypercortisolism. However, some caution should be exercised in the interpretation of studies of sleep patterns of a patient population compared to normal young adult controls. The mean age of the Cushing patient population was 36 whereas that of the control group was 23, and that of the acute and chronic corticosteroid group was 25. There was no adaptation period to the sleep laboratory prior to the one-night catheter recording for the patient group and considerable variability of both total recording and therefore total sleep time was obtained. Each of these factors separately and in combination might well alter the nocturnal sleep stage pattern and therefore the consequent GH secretory pattern.

## THE PATTERN OF PROLACTIN SECRETION DURING SLEEP AND WAKING IN MAN

A specific and sensitive radioimmunoassay for the measurement of human prolactin in plasma has been available for only a few years (80, 81). Therefore, the analysis of the 24-hr pattern of its secretion has only begun. Nevertheless, it has been clearly demonstrated that there is a relation to the sleep-waking cycle (82, 83). In all adult subjects studied a large increment in the plasma prolactin concentration takes place between 60 to 90 min after the sleep onset period at night and is generally followed by a series of even larger secretory episodes resulting in progressively higher plasma concentrations during the night with peak values occurring at the end of the sleep period, generally at 5 to 7 AM (Figure 1). Upon awakening, a rapid fall in concentration occurs, with plasma concentration reaching the lower daytime waking values by 10 AM. Although the pattern of release of prolactin during both waking and sleep is clearly episodic, the pattern differs from that of cortisol and GH in that the prolactin never falls to undetectable values in normal subjects. When the sleep time is shifted or the sleep-waking cycle is inverted, it has been shown that prolactin release is immediately dependent on the occurrence of sleep.and not on an inherent rhythmic secretion pattern, as in the case of the ACTH-cortisol system (84).

In a recent study (85), analysis of the relationship between the episodes of prolactin secretion during sleep and the non-REM–REM sleep stage cycle demonstrated that a sharp rise of prolactin takes place at the beginning of the non-REM period and then falls prior to the onset of the next REM period. Lowest concentrations are present during REM sleep and highest values occur in the middle of the non-REM period. They confirmed the very rapid decrease in prolactin concentration upon awakening and found no difference whether awakening took place from a REM or non-REM sleep stage. It has also been shown that prolactin is secreted in larger amounts during daytime naps (83).

The hypothalamic mechanism of control of prolactin is considered to be primarily via an "inhibitory" rather than a "releasing" factor. Prolactin concentrations are frequently quite elevated in patients with pituitary and hypothalamic tumors (86).

Like GH and the ACTH-cortisol hormones, prolactin will be released in response to a variety of stimuli. These include stress, hypoglycemia, suckling in postpartum women, strenuous exercise, and a number of psychotropic drugs. However, L-Dopa administered to man will consistently produce a decrease in prolactin secretion (87–89), whereas it characteristically stimulates GH secretion (90, 91). In patients with Parkinson's disease, the recurrent suppression of prolactin with each dose of L-Dopa did not prevent the nocturnal sleep release (92).

The application of these findings to clinical problems is just beginning. In a series of nine patients with diverse hyperprolactinemic conditions (five had a pituitary tumor, one had a hypothalamic tumor, and three had "functional" disorders with galactorrhea), seven had an absence of the normal sleep increase of secretion during the night (93). The abnormally high prolactin concentration throughout the 24-hr period was equally elevated during sleeping and waking periods. The morning waking sharp decrement in concentration found in normal subjects was absent in these patients, indicating that the hypothalamic inhibitory mechanism was not functioning normally. These findings as well as the similar 24-hr abnormalities in patients with Cushing's disease have led us to hypothesize that abnormal sleep-waking regulatory hypothalamic mechanisms may lead to a progressive hyperfunction of the pituitary gland for sustained and inappropriate periods of the day, and that this may lead to frank pituitary adenomas (28).

## THE RELATIONSHIP OF GONADOTROPIN HORMONE (LH AND FSH) SECRETION TO SLEEP—WAKING PATTERNS

The application of the 24-hr frequent plasma sampling technique to the gonadotropin system has been quite fruitful and has demonstrated that there are important sleep-waking relationships. Perhaps the most significant finding is that in normal pubertal boys and girls a major increment in plasma LH concentrations occurs in an episodic pattern during the nocturnal sleep period (93) (Figure 2). No sex difference was found. In prepubertal children this sleep LH augmentation was not found, and the values are in a low range of concentration. In general, a relation was noted between the number of LH episodes and the number of sleep cycles; the LH episode interval of 70–90 min closely matched the 80–90 min non-REM–REM cycle. The temporal relation to sleep stages suggests that LH secretion is initiated during non-REM sleep and terminates in relation to the REM sleep stage. As puberty proceeds, the sleep-related enhanced secretory episodes also occur during the waking portion of the 24-hr period so that by the completion of sexual maturation, the sleep and waking patterns are equivalent with respect to both the mean concentration and the episodic pattern. The sleep-augmented LH release has been shown to be associated with a corresponding increase in testosterone (94).

Acute inversion of the sleep-waking cycle in a group of pubertal boys demonstrated a concomitant and immediate shift of the augmented secretory pattern of LH and testosterone during daytime sleep (95). Although the mean LH and testosterone concentration was higher during daytime sleep as compared with the nocturnal waking values, the latter values were significantly greater than the daytime waking values obtained prior to the sleep-waking phase shift.

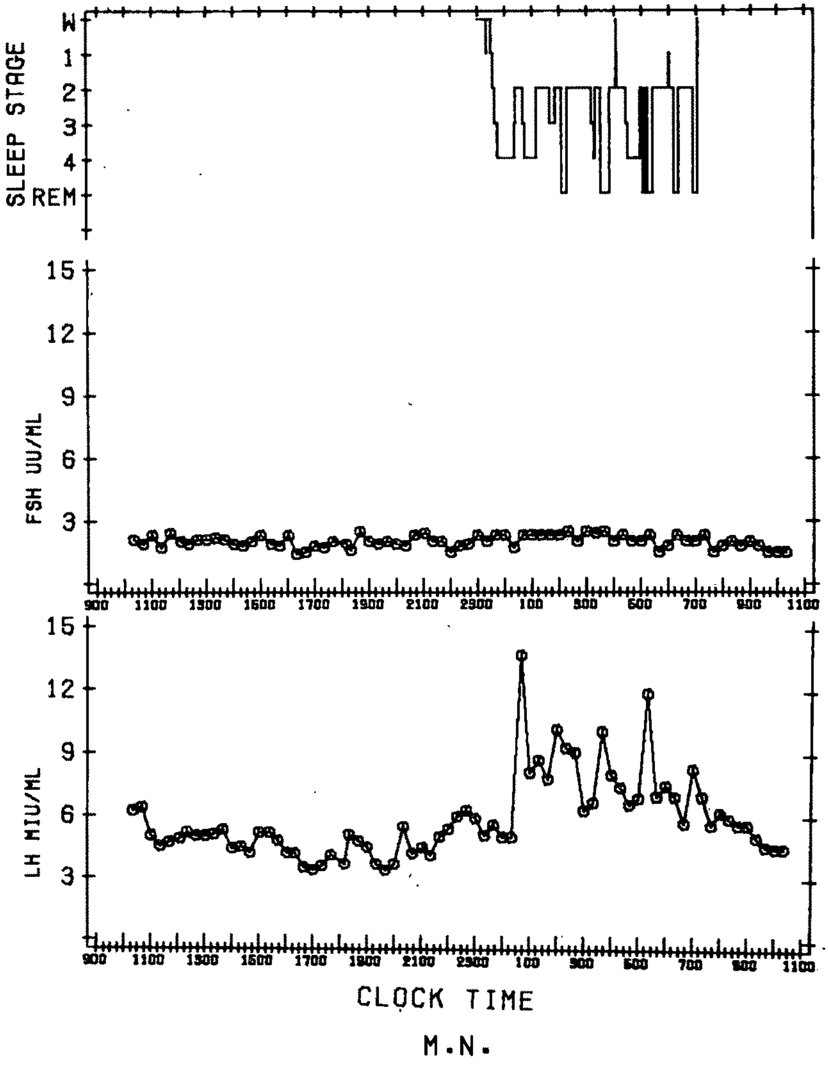

*Figure 2* Twenty-four-hour pattern of LH and FSH secretion in a pubertal boy, age 14. Sleep stages are defined by polygraphic recording.

These findings indicate that the relationship between CNS sleep processes and LH hormonal release via hypothalamic-pituitary control is the major mechanism for the initiation and subsequent maturation of sexual pubertal changes. In normal adult men, we found that LH was secreted episodically (approximately 12 episodes for a 24-hr period), but we were unable to demonstrate a 24-hr sleep-waking relationship (96). Rubin et al (97) reported that in men LH values were 14% greater during REM periods when compared to other sleep stages combined. Nankin & Troen (98) reported that LH serum values were highest in men between 3 and 7 AM during the sleep period compared with earlier evening values. In a recent report, concomitant measurement of luteinizing hormone-releasing factor (LRF) with LH in man indicated that there was an overall correlation between the two assays, but at times there was a discordant relationship (99, 100). A nocturnal rise in plasma testosterone in normal men has been found (101, 102) with a temporal relation to LH secretory episodes (103). This testosterone augmentation is probably of testicular origin since it can be dissociated from the cortisol-correlated pulses of androstenedione and dehydro-3-epiandrosterone (104). It is therefore possible that the enhanced secretion of testosterone during sleep may be due to local changes in testicular function, such as alteration in blood flow, related to autonomic nervous system changes (100, 103).

There has been a series of studies in sexually mature women investigating the 24-hr pattern of LH and FSH secretion during selected portions of the menstrual cycle. The gonadotropin hormones have been shown to be secreted in an episodic manner; however, the pattern, frequency, and amplitude differ depending on the phase of the cycle. During the early follicular phase, LH is secreted by a sequence of 10 to 15 episodes during the 24-hr period (105) (Figure 1). Instead of a major increment of LH during sleep such as is found in pubertal children, a significant decrease in the plasma LH concentration was found during the first 3 hr after sleep onset. When the onset of the first Stage 2 was used as reference point, a 33% decrease from the mean LH was found for the 3 hr of sleep. This was not found in an age-matched normal male group. Acute inversion of the sleep-waking cycle in women during the follicular phase demonstrated that this early sleep LH concentration decrease was present during the daytime sleep period as well (95). These findings suggest that transient inhibition of LH secretion occurs shortly after sleep onset and that the sequence of sleep Stages 2, 3, and 4 may be an important factor.

During the periovulatory phase of the menstrual cycle at the time of the LH "surge," large recurrent secretory episodes were present, superimposed on a progressive elevation of the baseline concentration (106). In three subjects, the presumptive onset of the LH surge appeared to take place in close proximity to the end of the nocturnal sleep period.

During the mid-and late luteal phases, a diminished frequency of secretory pulses has been found with an interval of 3–4 hr (107). Although pulsatile secretion of FSH is not as prominent as that of LH, similar changes were found during the early follicular and midcycle phases.

Coincidental pulses of FSH and LH are found in postmenopausal women and in patients with gonadal dysgenesis in which the feedback loop from ovarian steroids is absent (108).

The knowledge of these normative 24-hr patterns of gonadotropins has begun to be applied to clinical problems during the past few years. The measurement of 24 LH secretory patterns was done in a group of children with precocious puberty due to different causes (three with idiopathic, one with hypothalamic tumor, and two with congenital adrenal hyperplasia) (109). All six children had a sleep-related increased release of LH. The two boys with adrenal hyperplasia (ages 6 and 8.5 years) had increased amounts of adrenal androgens and therefore had triggered the pubertal LH CNS "program." However, in a 12-year-old girl with gonadal dysgenesis (Turner's syndrome) there was also an augmented secretion of LH and FSH during sleep (110). The 24-hr mean LH and FSH concentrations were markedly elevated, as expected, in the castrate range. These findings suggest that normal ovarian function may not be required to initiate the pubertal sleep-related gonadotropin secretion.

A patient with persistent postpartum amenorrhea and galactorrhea (Chiari-Frommel syndrome) was studied with 24 hr plasma sampling (every 20 min) for LH and prolactin hormone concentrations in association with polygraphic sleep recording (111). This was done before and after treatment with clomiphene citrate. A striking decrease in the concentration of LH and FSH occurred during the night in both sampling periods. The prolactin pattern demonstrated the characteristic concentration elevation during the night, although at higher than normal values. This reciprocal relationship of the nocturnal sleep patterns of gonadotropins and prolactin in this patient may indicate that there is an abnormality of a CNS neurotransmitter system linked to the sleep-wake cycle underlying this clinical syndrome.

A group of nine young women with moderately severe anorexia nervosa (24–39% below ideal body weight) and amenorrhea have been studied with the 24-hr frequent plasma sampling and sleep recording procedure (112). A pubertal-type augmented nocturnal sleep-related LH release was found inappropriate to the chronological age (17–23). The other five patients demonstrated a prepubertal-type pattern. Study of a larger series of women with other types of amenorrhea did not demonstrate the pubertal type of 24-hr LH pattern. One of the patients with anorexia nervosa was restudied after clinical remission and normalization of body weight showed a return to the normal adult-type 24-hr LH pattern. These data suggest that the amenorrheic defect in anorexia nervosa may be associated with a return to a pubertal-type "programmed" LH CNS pattern, but that this may be reversible when clinical remission occurs.

## CONCLUSION

It is now well established that hypothalamic-pituitary hormonal systems have temporal patterns of secretion that are clearly related to 24-hr sleep-waking activity in man. Each of the four hormonal systems (ACTH-cortisol, growth hormone, prolactin, and gonadotropin hormones) discussed in this report has its own temporal organization and response pattern to alterations of the sleep-waking cycle and no single principle or mechanism can explain these patterns. Previous concepts of constancy of blood levels in regard to closed-loop feedback control are challenged by data that demonstrate widely fluctuating hormone concentrations produced by episodic secretory activity.

[2] A second case with the Chiari-Frommel Syndrome has been studied by our group and a similar 24-hr pattern of LH and prolactin was found.

Cortisol and ACTH are clearly secreted in an episodic manner, with the episode frequency occurring on a 24-hr basis. The 24-hr cortisol pattern is relatively resistant to shift to the sleep-waking cycle and can be dissociated from sleep in spite of a highly correlative relationship under normal stable circadian conditions.

Growth hormone secretion at night is intimately related to sleep and closely associated with the specific sleep Stages 3-4, particularly in relation to the period immediately after sleep onset. In contradistinction to the cortisol pattern, the secretion of GH can be readily shifted by shifting the time of sleep.

Prolactin is also secreted in larger amounts during the night's sleep period, with initiation just following sleep onset and increasing in an episodic manner throughout the sleep period. There is an abrupt fall in concentration upon awakening in the morning. An immediate shift of this pattern occurs with a shift of sleep time.

The findings for gonadotropin hormones strongly suggest that in man, CNS sleep mechanisms are intimately involved in the initiation and maturation of normal pubertal hormonal changes. In pubertal boys and girls, there is an augmented release of LH with sleep at night and if sleep is shifted, the LH increment is also shifted. During the follicular phase of the menstrual cycle in sexually mature women, there appears to be a transient decrease in LH plasma concentration during sleep. Although the data in general do not support a clear sleep-waking relationship for LH in men, nevertheless there does appear to be a nocturnal increase in testosterone. In pubertal boys there is a clear increase in testosterone concentration accompanying the sleep rise of LH.

These 24-hr hormonal patterns have been applied to a wide variety of neuroendocrine clinical syndromes. These include Cushing's disease, primary aldosteronism, amenorrhea-galactorrhea syndromes, precocious puberty, gonadal dysgenesis, acromegaly, anorexia nervosa, and depressive affective disease. The findings from these clinical studies have begun to provide new diagnostic and mechanistic insight, and emphasize the potential usefulness of applying a chronobiologic analysis to the relations between 24-hr neuroendocrine events and the sleep-waking cycle in man.

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# VITAMIN K AND THE ORAL ANTICOAGULANT DRUGS[1]

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## INTRODUCTION

Vitamin K activates four proteins that clot blood. The oral anticoagulant drugs hinder the activity of vitamin K and thereby retard clotting. As this subject has never been reviewed in this series, I discuss in detail the discovery and physiology of vitamin K, its mechanism of action, the clinical pharmacology of the oral anticoagulants, and the interactions of those drugs with others.

## VITAMIN K

### *Historic Aspects*

Investigation of vitamin K began with the birds in 1929, when chicks fed a diet devoid of ether-soluble components bled to death (1). The blood was incoagulable (2), but responded to dietary addition of the ether extracts (3), which contained an unidentified substance called the Koagulations vitamin or vitamin K (4). The hemorrhagic diathesis resulted from a marked reduction in prothrombin (factor II) concentration (5). Vitamin K was discovered in green leafy plants, especially alfalfa (3), in bacteria (6), and even in the feces of vitamin K-deficient chicks (7). In 1939, vitamin K was isolated, identified, and synthesized in four laboratories (8–11), for which Dam and Doisy shared the Nobel prize in 1943 (12).

In man, it was observed that a similar bleeding disorder occurs when the delivery of bile to the bowel is hindered [e.g. obstructive jaundice (13) or a biliary fistula (14)] and that the bleeding can be corrected by ingestion of a source of vitamin K (15). Vitamin K deficiency also develops in patients with a variety of intestinal diseases (7); although the vitamin is abundantly present in the bowel, the disease prevents it from being absorbed (13). By 1941, obstructive jaundice in man was found analogous, both in the fundamental nature of the bleeding tendency (a lack of

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prothrombin) and the curative mechanism effected by vitamin K and bile, to the bleeding disease produced in experimental animals when the intestinal drainage of bile was prevented by a biliary fistula (15). These findings bridged the gap of knowledge between the hemorrhagic syndrome of chicks fed a vitamin K-deficient diet and the bleeding tendency in jaundiced patients (16).

Four basic factors are necessary to prevent vitamin K deficiency: (*a*) a normal diet containing the vitamin; (*b*) the presence of bile in the intestine; (*c*) a normal absorptive surface in the small intestine; and (*d*) a normal liver (17). Clinical states in which vitamin K intake or production may be inadequate include many otherwise normal newborns, breast-fed infants (18), infants on artificial formulas deficient in vitamin K (19), protracted antibiotic therapy (20), and long-term parenteral hyperalimentation without vitamin K supplementation (21). Malabsorption of vitamin K occurs in patients with obstructive jaundice (11), biliary fistula (13), pancreatic insufficiency (22), steatorrhea (15), chronic diarrhea (23), and in young infants with acute diarrhea (24). Failure of utilization occurs in patients with hepatocellular disease (25) and in premature infants with hepatic immaturity (21). Pharmacologic antagonism occurs with coumarin anticoagulants, salicylates, and anticonvulsant drugs (26).

In 1937, prothrombin was demonstrated in the cord blood of infants (27), and in 1939, vitamin K was found effective in treating many cases of hemorrhagic disease of the newborn (28). The fetus apparently synthesizes the vitamin K-dependent clotting factors with vitamin K transferred placentally. At birth, these factors are often depressed but usually not to hemorrhagic levels (29). After birth, an infant is generally given nothing by mouth for a few hours and then glucose and water feedings for the first 24 hr. Because of this lack of vitamin K, the prothrombin time prolongs further and hemorrhage may ensue (30). In the normal neonate receiving cow's milk formula, a normal prothrombin time develops because the bowel becomes colonized with bacteria that synthesize vitamin K (18). Conversely, in the breast-fed infant, hemorrhage may develop because breast milk is sterile and low in vitamin K content (31).

Infantile gastroenteritis frequently causes hemorrhage because the infant has marginal stores of vitamin K, no intake of the vitamin when changed to intravenous fluids, reduced bacterial synthesis of the vitamin because of antibiotic administration, and reduced absorption of the vitamin because of rapid transit time (29). Management includes a determination of the prothrombin time and parenteral administration of 1 mg of vitamin $K_1$. These same management principles apply to all infants facing elective surgery because severe hemorrhage can occur in otherwise normal infants undergoing even minor surgery (32). Vitamin K deficiency has been reported even in hospitalized adult patients who have had recent surgery, poor food intake, or antibiotic therapy (33).

### *Diverse Forms*

Several kinds of vitamin K exist. Vitamin $K_1$ or phytonadione (2-methyl-3-phytyl-1,4-naphthoquinone) was initially isolated from the photosynthetic regions of higher plants and has a 20-carbon side chain (K-4) attached to the third carbon of the naphthoquinone nucleus (34). Vitamin $K_2$ or menaquinone was initially isolated from putrefied fish meal and bacteria and has a 20 (MK-4) to 60 (MK-12) -carbon side chain attached to the naphthoquinone nucleus (35). The high molecular weight forms of vitamin $K_2$, especially MK-7 in man, are the storage forms of vitamin K in man (36) and animal (37). Vitamin $K_3$ or menadione is a synthetic, fat-soluble form of the vitamin without any side chain attached to the third carbon of the naphthoquinone nucleus (K-0), from which water-soluble phosphate (Synkayvite®) and sulfate (Hykinone®) salts were prepared (38). Vitamin $K_5$ or Synkamin® is a synthetic form of the vitamin with an amino group substitution that has been used primarily in obstetrics prophylactically to prevent hemorrhagic disease of the newborn (39).

Reduced activity of clotting factors II, VII, IX, and X, but not other clotting factors, results only from vitamin K deficiency or administration of oral anticoagulants (Figure 1). The response to vitamin $K_1$ and $K_3$ may be used to differentiate the hypoprothrombinemia of these two states (40). Vitamin K deficiency responds

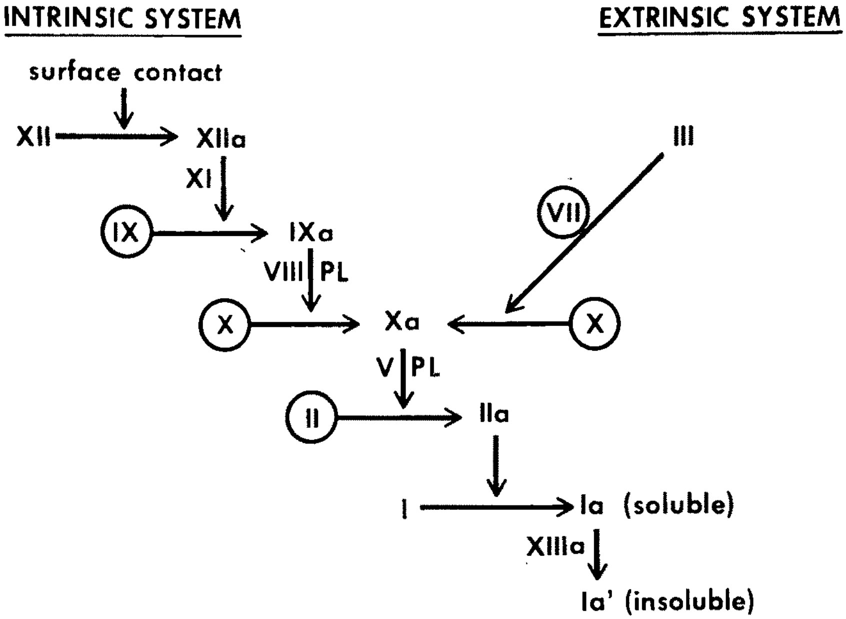

*Figure 1* Reaction sequence for the intrinsic and extrinsic systems of blood coagulation. Both systems lead to the formation of a urea-insoluble fibrin clot (Ia'). The four vitamin K-dependent clotting factors are encircled. Ionic calcium, which facilitates most of the reactions depicted, has been omitted for the sake of clarity. Platelet phospholipid (PL) provides the surface on which factors II and X, and perhaps factor IX, are activated. Tissue thromboplastin (III) may provide the surface for the "activation" of factor VII. Factor I is fibrinogen; Ia, soluble fibrin monomers; II, prothrombin; IIa, thrombin; V, proaccelerin; VII, proconvertin; VIII, antihemophilic globulin; IX, PTC or Christmas factor; X, Stuart-Prower factor; XI, PTA or plasma thromboplastin antecedent; XII, Hageman factor; and XIIIa, activated form of fibrin stabilizing factor. [Reproduced from (89).]

promptly to a small dose of a water-soluble preparation of vitamin $K_3$ (Hykinone) (41), whereas the hypoprothrombinemia of oral anticoagulant therapy responds poorly to vitamin $K_3$ and requires large doses of vitamin $K_1$ for its correction (42).

## *Molecular Pharmacology*

Vitamin K action at the molecular level was suggested in 1963 from kinetic analysis of patients on oral anticoagulants in whom the formation of a competitive inhibitor was predicted to be a precursor protein to prothrombin (43). By 1968, these precursor proteins, identified by means of antibodies in human (44) and later bovine (45) plasma, were found to be antigenically similar to prothrombin but lacked the ability to adsorb barium salts or to bind calcium. Because prothrombin is a glycoprotein, the glycosylation step was tested as the vitamin K-sensitive site. The incorporation of carbohydrate by, and the carbohydrate content of, prothrombin were not altered by oral anticoagulants (46), which indicated that the carbohydrate portion of prothrombin was not important in its procoagulant function.

Earlier experiments with the protein inhibitor actinomycin D suggesting a vitamin K effect on DNA transcription (47) were refuted, particularly when cycloheximide did not prevent prothrombin release into the plasma (48). This failure to block amino acid incorporation also suggested that vitamin K did not facilitate the synthesis of protein but acted at a postribosomal site (49). Recent work has concentrated on posttranscriptional events to determine whether vitamin K regulates the rate of synthesis of prothrombin (50) or converts a precursor molecule to prothrombin (51).

## *Mechanism of Action*

The bovine form of the precursor protein to prothrombin has been studied extensively (52). The protein is chemically indistinguishable from prothrombin except it cannot be adsorbed by insoluble barium salts, a characteristic of the four vitamin K-dependent clotting factors, and it cannot bind calcium ions in solution. This lack of calcium binding prevented the activation of prothrombin at a hemostatically effective rate because calcium is required to hold prothrombin onto a phospholipid surface during its normal activation to thrombin by the activated form of factor X (Xa) (53). Prothrombin was also activated to thrombin by specific proteases of snake venoms, particularly that from *Echis carinatus* (54). These data indicate that the precursor protein contained an intact thrombin portion of the prothrombin molecule but was inactive because of a defect in activation (51). The vitamin K-sensitive step was shown to be the modification of amino acid residues on the precursor to form the calcium binding site (55). Vitamin K induced the carboxylation of ten glutamyl residues from the amino terminal end of the precursor protein to form several pairs of a unique amino acid, $\gamma$-carboxyglutamic acid (56). This step converted the precursor protein to prothrombin and provided the calcium-binding site of prothrombin. Vitamin K is probably essential for the carboxylation of factors VII, IX, and X as well because it induced the carboxylation of glutamyl residues in factor X (57). Further proof of this conversion of precursor protein to prothrombin without the synthesis of new protein occurred when biologically active prothrombin was formed in vitro after addition of vitamin K to postmitochondrial supernatant of vitamin K-deficient rats (58).

### *Receptor Theories*

Several theories for the mechanism of the procoagulant effect of vitamin K and the anticoagulant effect of coumarin drugs exist:

1. Vitamin K and coumarin drugs interact with a ribosomal regulatory protein in the de novo synthesis of prothrombin (59).
2. Vitamin K and coumarin drugs bind to the same or different sites on a receptor protein either competitively or noncompetitively (60).
3. Coumarin drugs act by blocking a normal transport pathway of vitamin K, which can be bypassed with large doses of the vitamin diffusing via an alternate nonwarfarin-sensitive pathway (61).
4. Coumarin drugs block the conversion of biologically inactive vitamin K oxide (the 2,3-epoxide of vitamin K), a normal metabolite, back into active vitamin K (62).
5. Vitamin K functions in the metabolic conversion of a precursor protein to prothrombin that is blocked by warfarin (51).

Note that these theories are not dependent upon the true vitamin K-sensitive step, but rather assume the existence of a receptor protein for the vitamin that is somehow antagonized by the coumarin drug (51). Warfarin does not alter the gross hepatic uptake of the vitamin or its subcellular localization (63).

A monogenic trait for tolerance to oral anticoagulants has been found in man and rat (64–66). In both species, the metabolism of the drug is normal but the requirement for vitamin K is high (67–69). These data suggest that the mutation is of a protein that binds both vitamin K and coumarin. While the rats are resistant to coumarin and indanedione anticoagulants, they respond normally to a direct antagonist of the vitamin, chloro-$K_1$ (replacement of the 2-methyl by a chloro group in vitamin $K_1$) (70). Although the various theories can be stretched to cover both normal and resistant conditions, the data are best explained by a summation of several of the theories. While vitamin K is undergoing conversion to vitamin $K_1$ epoxide, it converts a precursor protein to prothrombin by facilitating the addition of the calcium-binding site to the protein (Figure 2). This step is chloro-K sensitive

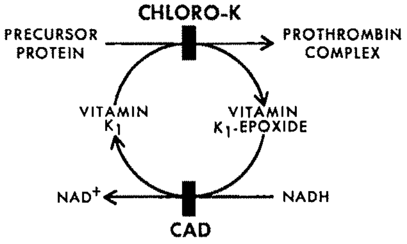

*Figure 2* Theoretical interrelationships of vitamin K metabolism and anticoagulant substances. Vitamin $K_1$ converts a precursor protein of prothrombin while undergoing conversion to inactive vitamin $K_1$ epoxide, a step that is chloro-K sensitive and warfarin insensitive. Vitamin $K_1$ is regenerated by a reduced nicotinamide adenine dinucleotide system (NADH), a warfarin-sensitive step. CAD represents any coumarin anticoagulant drug.

and warfarin insensitive, which explains the rodenticidal effect of chloro-K even in the warfarin-resistant rat. Vitamin K is then regenerated from vitamin K epoxide by a reduced nicotinamide adenine dinucleotide (NADH) system, a warfarin-sensitive step. This locus presumably is the mutation site for the development of hereditary warfarin resistance in man and rat.

## ORAL ANTICOAGULANT DRUGS

### *Clinical Preparations*

Many oral anticoagulant drugs have been synthesized (71). Ethyl biscoumacetate (Tromexan®) is poorly controlled clinically because the half-life ($T_{½}$) in plasma is too short, only 2 hr (72). Phenprocoumon (Liquamar®) has equal difficulties because the 156-hr $T_{½}$ renders the treatment of toxic overdose dangerous (73). Bishydroxycoumarin (Dicumarol®) is poorly controlled clinically because of its incomplete gastrointestinal absorption and dose-dependent $T_{½}$ in plasma (74). No indanedione drug (e.g. phenindione) should ever be used clinically because of the significant incidence of cutaneous and hepatic toxicity (75). Acenocoumarin (Sintrom®) is potentially hazardous for long-term therapy because of the nitrobenzene moiety (71). Sodium warfarin (Coumadin®, Panwarfin®) is popular and well controlled clinically because of rapid and complete gastrointestinal absorption, a $T_{½}$ of 36 hr that is not dose dependent, and the absence of significant direct toxicity (76).

### *Initiation of Therapy*

Formerly, warfarin therapy was initiated with a large loading dose of 40–60 mg on the first day. Rapid action ensued with this dose because synthesis of the vitamin K-dependent clotting factors was completely inhibited (77). However, initiating therapy with a large loading dose was dangerous because excessive hypoprothrombinemia and hemorrhage can occur (78), particularly in patients who are elderly, septic, debilitated, suffering from liver disease or congestive heart failure, or who have recently undergone major surgery (79). The 36-hr $T_{½}$ of warfarin in plasma results in bodily accumulation of drug when doses are administered every 24 hr (80).

Currently, no loading dose of warfarin is recommended because a comparative study revealed that the one-stage prothrombin time reached the therapeutic range in 5 days with 10 mg warfarin daily, 3 days with 15 mg warfarin daily, and 1 day with a large loading dose (81). The earlier achievement of the therapeutic range with the large loading dose over the 15-mg dose resulted solely from the more rapid decline in factor VII (proconvertin) activity, because the rates of decline in the activities of factors II, IX, and X were not significantly different (81). Because factor VII is not involved in the intrinsic system of blood clotting, it was concluded that prophylactic induction of antithrombotic therapy with oral anticoagulants does not require a large loading dose.

### *Optical Enantiomers*

All the sodium warfarin used clinically is a racemic mixture composed equally of two optical enantiomers: R(+)-warfarin and S(–)-warfarin (82). S(–)-Warfarin has a hypoprothrombinemic effect four to five times greater than R(+)-warfarin in both rat (83) and man (84). The stereospecificity of some drugs' interaction with warfarin has been demonstrated by administration of separate optical enantiomers of warfarin (84) and by careful study of the metabolic products of racemic warfarin (85). Further studies with alternate $^{13}C$-labeling of one optical enantiomer of racemic warfarin should clarify the stereospecificity of drug interactions with anticoagulants. If several drug interactions occur only with the more potent (–)-warfarin, these interactions could be avoided by the clinical use of (+)-warfarin alone (86).

## DRUG INTERACTIONS WITH ORAL ANTICOAGULANTS

The oral anticoagulants are ideal agents for evaluating drug interactions because of the ready detection of bleeding and the ease of measuring the blood levels of drug and of quantitating the effect on the one-stage prothrombin time (87). Drug interactions have two general mechanisms: pharmacokinetic and pharmacodynamic. The pharmacokinetic mechanism is one in which one drug affects the metabolism of a second drug. The pharmacodynamic mechanism is one in which the pharmacologic effect of the second drug is altered.

### *Pharmacokinetic Interaction*

DECREASED GASTROINTESTINAL ABSORPTION *Cholestyramine* resin (Questran®) decreases blood levels of warfarin and lessens the hypoprothrombinemic effect in man when cholestyramine and warfarin are both administered orally (88). The mechanism for this interaction of reduced gastrointestinal absorption was proved when unchanged warfarin was found in the stool of normal subjects only during the administration of cholestyramine (89). *Barbiturates* increase the malabsorption of a poorly absorbable oral anticoagulant, Dicumarol, but have no effect on the absorption of a well-absorbed anticoagulant, warfarin (90). *Antacids* have no effect on the absorption of oral anticoagulants (88). The rate of absorption of oral anticoagulants correlates with the rate of dissolution of the tablets: rapid for warfarin and slow for Dicumarol (74). The dosage form of warfarin tablets makes surprisingly little difference as both the absorption rate (91) and dissolution rate were equally rapid both for warfarin acid, the rodenticidal form, and for warfarin sodium tablets, the clinical form (92). The effect of the pharmaceutical form of a dose of a drug on its appearance in the general circulation, called bioavailability, has been emphasized recently in therapeutics. However, reliance on blood levels of drug alone without correlation with the biologic effect can be misleading and dangerous (93).

FASTER CLEARANCE *Barbiturates* reduce the hypoprothrombinemia of oral anticoagulants, which correlates directly with lower blood levels of anticoagulant because of more rapid clearance of drug (94). This effect was exaggerated with Dicumarol because barbiturates additionally interfere with its gastrointestinal absorption (95). Most of the other sedatives and hypnotic drugs, including chloral hydrate, have no real effect on long-term therapy with oral anticoagulants (96, 97).

The antibiotic *rifampin* causes a severe reduction in both the blood levels of warfarin and the hypoprothrombinemia in man (98). The effect of rifampin is so profound that during long-term anticoagulant therapy, the blood level of warfarin often falls to zero and the prothrombin activity returns to 100% (99). Because rifampin induces adrenal crises in patients on corticosteroid therapy (100) and breakthrough pregnancy in women on oral contraceptives (101), induction of hepatic enzymes by this antibiotic seems likely (102).

SLOWER CLEARANCE *Disulfiram* (Antabuse®) slowed the clearance of warfarin from the body, which leads to a longer plasma $T_{½}$ of the anticoagulant and a greater hypoprothrombinemic effect (103). Like its effect on ethanol, disulfiram probably inhibits the hepatic metabolism of warfarin. The effect is stereospecific in that it occurs only with the (–)-warfarin enantiomer and can be avoided in therapeutics with the (+)-warfarin enantiomer (86).

*Metronidazole* (Flagyl®), which has an antabuse-like effect, also slows the metabolism of warfarin and enhances the hypoprothrombinemic effect (86). The stereospecificity of the interaction is identical to that of disulfiram, present with (–)-warfarin and absent with (+)-warfarin (86).

*Ethanol* allegedly augmented the hypoprothrombinemia of long-term therapy because of the frequency of bleeding episodes in alcoholic patients. In one study, a large single dose of alcohol or an alcoholic beverage had no effect on the one-stage prothrombin time of patients on long-term therapy, either 1 hr (104) or 24 hr later (105). An increased rate of warfarin clearance from hepatic enzyme induction was found in alcoholic patients (106) and two case reports showed a reduced response to Dicumarol during chronic consumption of ethanol (107). Anticoagulant therapy is contraindicated in alcoholic patients, not because of a drug interaction but because of their sporadic unreliability as patients (108).

TRANSPORT AND BINDING TO PLASMA ALBUMIN *Phenylbutazone* (Butazolidin®) can cause severe hemorrhagic episodes during anticoagulant therapy by its effect on platelet aggregation, its induction of peptic ulceration, and its augmentation of hypoprothrombinemia (109). Phenylbutazone displacement of albumin-bound warfarin in vitro correlates with reduced plasma levels of total warfarin in vivo (110), and direct measurement of increased concentration of free warfarin in vivo after phenylbutazone administration confirmed the displacement (85). However, the same investigators also found evidence for prolongation of the (–)-warfarin $T_{½}$ by phenylbutazone (85), which was recently correlated with hypoprothrombinemic augmentation by this enantiomer (86). As it caused no significant change in hypoprothrombinemia, the interaction can be avoided in therapeutics with the use of the R(+)-warfarin enantiomer (86).

Two patients with the nephrotic syndrome and severe hypoalbuminemia required an increased daily dose of warfarin, 25[2] and 40 mg (111). Both the rapid clearance of warfarin and the relative resistance to the anticoagulant in these patients may have resulted from the reduced albumin binding in severe hypoalbuminemia (112).

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[2] Rapaport, S. I. Personal communication, 1969.

EXCRETION *Diuretics* reduce the response to oral anticoagulants (113). The theory that diuretics prevent hepatic accumulation of anticoagulant by augmenting renal elimination of the coumarin drug was refuted when no significant changes occurred in the plasma level of drug and no unchanged warfarin was found in the urine during administration of chlorthalidone (Hygroton®) (114). Chlorthalidone administered during initiation of warfarin therapy resulted in a highly significant loss of plasma water accompanied by increased clotting-factor activity. It was concluded that diuretics lessen therapeutic hypoprothrombinemia by concentrating clotting factors in plasma in normal subjects and by reducing hepatic congestion in patients with heart failure (114).

### *Pharmacodynamic Interaction*

VITAMIN K DEFICIENCY *Sulfonamide and antibiotic drugs* are said to potentiate oral anticoagulants by producing vitamin K deficiency from bowel "sterilization" (115). In animal experiments sulfonamides simulated the action of oral anticoagulants, but the postulated mechanism—reduction of intestinal bacteria—was not verified bacteriologically (116). Although one study with oral antibiotics administered to patients on long-term anticoagulant therapy significantly reduced the activity of the Prothrombin and Proconvertin test in some patients (117), several other studies failed to show any augmentation of the one-stage prothrombin time (69, 118–120). Thus sulfonamide and antibiotic drugs have little effect on anticoagulant therapy, unless both dietary and intestinal sources of vitamin K are reduced simultaneously (121). During long-term anticoagulant therapy the hypoprothrombinemia was augmented by a vitamin K-free diet (118), reduced by a vitamin K-enriched diet (122), and not altered by large doses of neomycin (118).

PRODUCTION OF CLOTTING FACTORS Administration of phenidione to patients with liver disease results in a greater increase in hypoprothrombinemia than in normal subjects (123). A pharmacokinetic interaction of liver disease was ruled out by the finding of a normal $T_{1/2}$ of the anticoagulant in these patients (124). Patients with congestive heart failure have augmented hypoprothrombinemia during anticoagulant therapy as hepatic congestion develops (125), which lessens after corrective cardiac surgery and relief of congestion (126). Development of acute viral hepatitis (127) or carbon tetrachloride intoxication (128) during long-term anticoagulant therapy resulted in excessive hypoprothrombinemia and hemorrhagic phenomena. Administration of vitamin K to patients with liver disease has been reported as effective, ineffective, and even harmful (129). These conflicting results are not unexpected, as many hemostatic abnormalities occur in patients with liver disease, i.e. defects of platelets or blood vessels and deficiencies of many clotting factors (I, II, V, VII, IX, X, XI, and XIII) (130).

Atromid®, a hypolipidemic agent containing the drug *clofibrate* and the hormone androsterone, augmented the hypoprothrombinemic effect of oral anticoagulants and is associated with frequent hemorrhagic episodes (131). All the above effects occurred with a preparation containing only clofibrate (Atromid-S®) (132). Clofibrate reduces platelet adhesiveness and epinephrine-induced platelet aggregation (133). Addition of clofibrate to warfarin therapy augments depression of the one-stage prothrombin activity and the activity of clotting factors II and X but has no effect on the blood levels of total warfarin (133). Thus the hemorrhagic complications of combined warfarin and clofibrate therapy result not from a pharmacokinetic interaction of the two drugs but from a combined hemostatic defect of reduced platelet function and more rapid turnover of the vitamin K-dependent clotting factors. Because anticoagulant augmentation also occurs with other hypolipidemic substances, D-thyroxine (134) and C-17-alkylated derivatives of testosterone (135), these substances may induce an alteration in the metabolism or activity of fat-soluble vitamin K.

*Hypermetabolic states* increase the responsiveness to oral anticoagulant drugs. This enhancement occurs in rats with induced fever (136) or hyperthyroidism (137) and in man with thyrotoxicosis (138). Conversely, myxedematous patients have an increased dose requirement for oral anticoagulants that disappears after treatment with L-triiodothyronine (139). The turnover rate of the vitamin K-dependent clotting factors after administration of oral anticoagulants increases threefold in patients with either hyperthyroidism or fever compared with control subjects (140). The reduced dose requirement for oral anticoagulants in hypermetabolic states results from an increased catabolism of the vitamin K-dependent clotting factors (141).

MUTATION OF RECEPTOR SITE Two human kindreds (65, 66) and rats in many geographic loci (142) have marked resistance or tolerance to oral anticoagulants that was inherited as an autosomal dominant trait (Figure 3). As discussed earlier, for both species the metabolism of the drug is normal but the requirement for vitamin K is high. It has been suggested that the mutational step is a reduced affinity of the receptor protein for both the vitamin and the drug (68, 69).

PLATELET FUNCTION *Acetylsalicylic acid* or aspirin has a profound effect on platelets (143). In one study, the cutaneous bleeding time was unaffected by anticoagulants alone, slightly prolonged by aspirin alone, and significantly prolonged by aspirin and anticoagulant administered simultaneously (144). Aspirin prolongs the bleeding time in patients with hemophilia or Von Willebrand's disease, which share with anticoagulant therapy an impairment of thrombin formation (145). Platelet plugs are formed primarily by collagen-induced aggregation of platelets and are stabilized by thrombin-induced formation of fibrin (146). When the former mechanism is unimpaired, as in patients receiving anticoagulant therapy alone or with severe hemophilia and no aspirin, the bleeding time is normal (114). The effect on platelet aggregation of anticoagulant and aspirin administered together is identical to that of aspirin alone: inhibition of collagen- and epinephrine-induced aggregation of platelets, inhibition of adenosine diphosphate (ADP) release from platelets, and a normal response to platelet aggregation on addition of extrinsic ADP (143). When 11 normal subjects on long-term anticoagulant therapy were given 6 aspirins a day (1.95 g), augmentation of the one-stage prothrombin time occurred in 4 of the subjects but not in the 2 subjects who had mild hemorrhagic phenomena (114). Thus, when platelet aggregation is impaired by aspirin in the presence of decreased

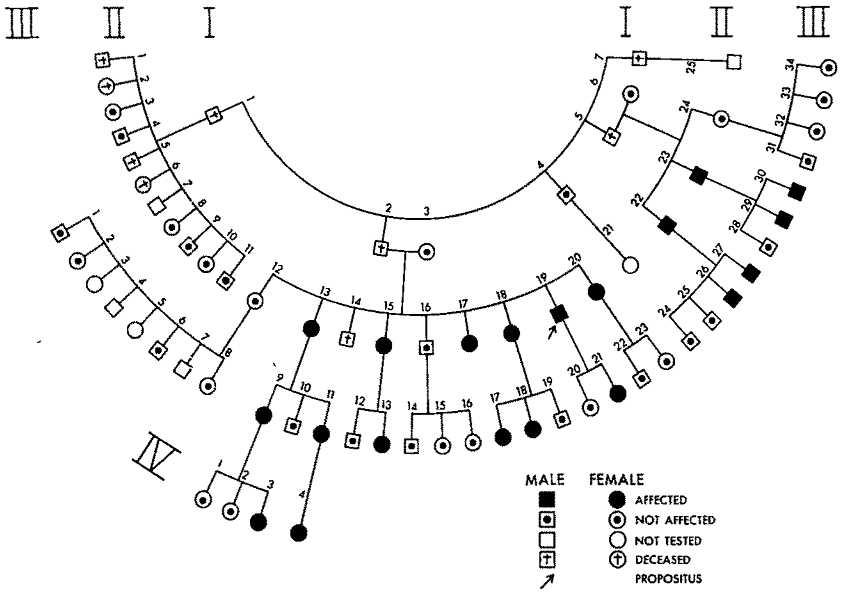

*Figure 3* The pedigree of warfarin-resistant kindred McC. Resistant members occurred in three generations, about half the members tested were found to be resistant, and the resistance occurred in and was transmitted by both male and female members. Of the eight resistant parents in generation II, ten of the nineteen children in generation III were resistant, whereas of the three normal parents in generation II, none of the eleven children of generation III were resistant. Of the two resistant parents in generation III, two of the four children in generation IV were resistant. Male-to-male transmission of the resistance occurred in four sons (III-26, III-27, III-29, and III-30) from two fathers (II-22 and II-23). These data are indicative of an autosomal dominant trait transmitted as a single gene effect. [Reproduced from (89).]

thrombin production, as in patients with severe hemophilia or on anticoagulant therapy, the bleeding time becomes quite prolonged and hemorrhage may occur.

DIRECT EFFECT ON PROTHROMBIN TIME Administration of ***heparin*** complicates oral anticoagulant therapy because of the combined effect on the one-stage prothrombin time (147). If parenteral heparin is discontinued and the oral anticoagulant continued at the previous dosage level, the prothrombin time will shorten toward normal values because the dosage of the oral drug itself was not sufficient (148). Heparin has less effect on the Prothrombin and Proconvertin test, probably because the test plasma is diluted tenfold (149).

## SUMMARY

The evolution of vitamin K, from a dietary deficiency in birds to a postribosomal modifier of prothrombin in man, has been a fascinating scientific saga. Its antivitamin, the oral anticoagulant drugs, has been a powerful probe both of vitamin K action and of drug interactions. These agents have emerged from a limbo of clinical therapeutics to become a light of human pharmacology.

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# MULTIPLE ENDOCRINE NEOPLASIA TYPE II[1]

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## INTRODUCTION

The syndromes characterized by the occurrence of several tumors involving various endocrine organs are referred to as multiple endocrine neoplasias. Generally, two types occur clinically, and both appear to be inherited as Mendelian dominant traits. In the first, termed multiple endocrine neoplasia type I (MEN-I) (1), there is an association of tumors involving the parathyroids, pancreatic islets, and pituitary gland. This syndrome is frequently accompanied by a fulminant peptic ulcer diathesis (Zollinger-Ellison syndrome). In multiple endocrine neoplasia type II (MEN-II) (2), medullary carcinoma of the thyroid gland (MCT), pheochromocytoma(s), and parathyroid hyperplasia are associated. It has been suggested (3) that the MEN-II syndrome can be attributed to a defect in a single-cell system, originating in the neural crest and representing a familial chromaffinomatosis.

Medullary carcinoma of the thyroid gland was described as a distinct clinical and pathological entity by Hazard (4) in 1959. As first proposed by Williams (5), this tumor arises from the parafollicular or C-cells of the thyroid gland. Several investigators (6–8) have demonstrated that MCT synthesizes and secretes the hormone thyrocalcitonin (TCT). In addition to the secretion of TCT, MCT has on occasion been shown to secrete a variety of other substances including ACTH (9), histaminase (10), prostaglandins (11), and serotonin (12).

Approximately 30% of patients with MCT have an associated diarrhea. Serotonin, prostaglandins, and TCT have all been proposed as agents that provoke this

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symptom; however, its etiology remains uncertain. TCT has been shown to increase the intestinal secretion of water and electrolytes when administered to normal subjects. The dosages of TCT required for this effect produce plasma TCT levels equivalent to those seen in many patients with MCT (13).

## CLINICAL PRESENTATION

Usually in MEN-II, the MCT is diagnosed first. Patients seek medical advice because of a lump in the neck or, less frequently, because of hoarseness or symptoms related to metastatic disease. Occasionally patients are symptomatic from functional pheochromocytomas, and a diagnosis of MCT is made subsequently. It is unusual for signs or symptoms of hyperparathyroidism to initiate the diagnosis.

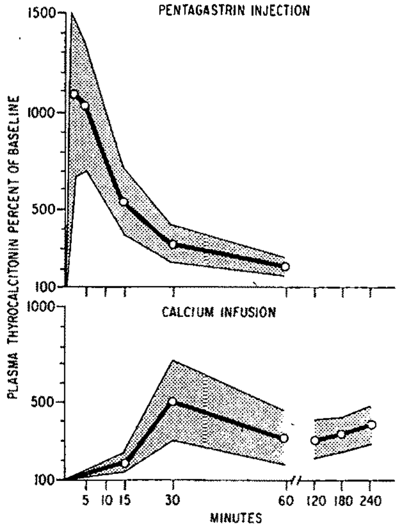
A genetic variant of MEN-II has been described in which patients with MCT and pheochromocytomas have associated multiple mucosal neuromas or neurofibromas, intestinal ganglioneuromatosis, characteristic facies, and a Marfanoid habitus (14). Patients with this variant can often be diagnosed in childhood because the characteristic facies and mucosal neuromatosis are apparent at a very early age.

## DIAGNOSIS

In 1968 several investigators (6–8) almost simultaneously demonstrated that MCT synthesizes and secretes TCT. This provided a rather unique clinical situation in which TCT was thought to serve as a marker for medullary thyroid carcinoma. Subsequently it has been shown that virtually all patients with MCT have elevated levels of TCT detectable in the peripheral blood. Other investigators have reported TCT to be elevated in various patients with disease states, including thyroiditis (15), the Zollinger-Ellison syndrome (16), oat cell carcinoma (17), and other nonthyroid tumors (18). This does not, however, detract from the usefulness of TCT measurement in patients suspected of having MCT, especially in kindreds with MEN-II where family members of an affected parent or sibling are at risk. It has been demonstrated in patients with MEN-II that an elevated level of TCT can often be detected before clinical disease is apparent. Several patients have been reported (19, 20) in whom an elevated TCT was the only clue to diagnosis, there being no thyroid disease palpable clinically or demonstrable on radioactive thyroid scanning.

Melvin & Tashjian (19) demonstrated that the infusion of calcium ion into MCT patients caused a marked elevation in TCT secretion, thus increasing the sensitivity of that hormonal measurement as a diagnostic test. Subsequently, Wolfe and associates (21) described two sisters who had small but progressive increases of serum TCT in response to calcium infusion. The thyroid glands were removed and subsequent pathologic examination revealed C-cell hyperplasia, a supposedly premalignant state.

Cooper et al (22) had demonstrated in pigs that the infusion of pentagastrin markedly stimulated the release of TCT. Subsequently, Hennessy and associates (23) demonstrated that pentagastrin was a more potent stimulator of TCT release in MCT patients than was calcium infusion. Furthermore, the peak of TCT response occurred 1–5 min following pentagastrin injection, as compared to 30 min following calcium infusion (Figure 1). In a subsequent study of a family with MEN-II, Hennessy et al (24) and Wells et al (25) demonstrated the superiority of pentagastrin stimulation in the early diagnosis of MCT. They described a total of five patients who had no elevated levels of TCT in response to calcium infusion [15 mg $kg^{-1}$ 4 $hr^{-1}$]. Four of the patients were children and all had elevated levels of TCT in the peripheral blood following stimulation with pentagastrin (0.5 mg $kg^{-1}$) (Figure 2). At surgery all of these children had very small tumors confined to the thyroid gland. The fifth patient was an adult who had no detectable elevation of TCT in the peripheral blood following calcium or pentagastrin stimulation. An elevated level of TCT, however, was detected in plasma sampled from a selectively catheterized inferior thyroid vein during pentagastrin stimulation. At subsequent surgery this patient was found to have C-cell hyperplasia. Selective venous catheterization with or without pentagastrin stimulation has also been described as a sensitive method of detecting foci of metastatic disease (25, 26).



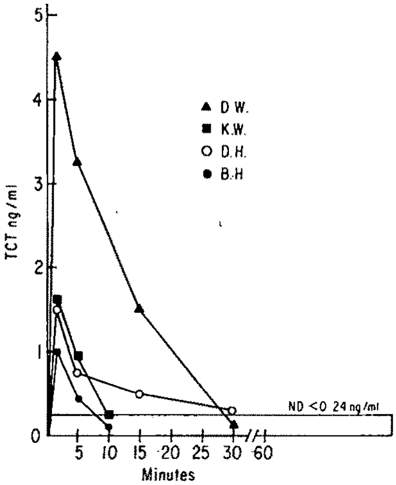


*Figure 1* (*left*) The combined responses of seven patients with elevated baseline levels of plasma TCT to pentagastrin injection and calcium infusion. Responses are expressed as the percentage of increase in immunoreactive TCT above initial baseline levels. Open circles and solid lines represent the mean responses and the shaded areas indicate the range of the standard errors. (Courtesy of the *Journal of Clinical Endocrinology and Metabolism.*)

*Figure 2* (*right*) The response of TCT in the peripheral plasma of four MEN-II patients (D.W., K.W., D.H., and B.H.) following stimulation with pentagastrin. None of these patients had thyroid neoplasms detected clinically or by radioactive thyroid scanning. None of these patients had an elevation of TCT in the peripheral plasma following calcium infusion. Shaded area represents limits of sensitivity of the radioimmunoassay (0.24 ng $ml^{-1}$). (Courtesy of *Annals of Surgery.*)

Screening patients for the presence of pheochromocytoma(s) should consist of collecting 24-hr urine samples for epinephrine, norepinephrine, metanephrine, normetanephrine, or vanillylmandelic acid.

Parathyroid hyperplasia occurs in approximately 60% of patients with MEN-II. Usually patients have grossly enlarged parathyroid glands and are hypercalcemic, but sometimes the only finding suggestive of parathyroid hyperplasia may be an increased ratio of chief cells to fat cells (19). The relationship between MCT and parathyroid hyperplasia is controversial. There are those who feel that the parathyroid hyperplasia is a reactive phenomenon secondary to the high levels of circulating TCT; however, others feel that the parathyroid hyperplasia is not secondary to excess plasma TCT, but occurs as a distinct part of the clinical entity. Hypocalcemia rarely occurs in patients with MCT, even though some individuals develop extraordinarily high levels of plasma TCT.

## TREATMENT

The treatment of MEN-II is primarily surgical, and every attempt should be made to diagnose the disease, especially the MCT, in its preclinical state. As mentioned above, this can be done most efficiently by utilizing serial pentagastrin stimulation tests in patients at risk. Should the diagnosis of MCT be made in a family member, then the presence of a concomitant pheochromocytoma must be ruled out before the person is subjected to neck exploration. The operation of choice for MCT in MEN-II is at least total thyroidectomy. The thyroid carcinoma is bilateral in virtually all patients with MEN-II and a lesser procedure will almost certainly result in recurrence of the malignancy. If positive lymph nodes are detected at the time of initial operation, then a neck dissection should be performed on the involved side. Prophylactic radical neck dissection is not advised in patients with this disease, especially if the diagnosis is made early, before the tumor is palpable clinically. Pathological examination of removed tissue will reveal the characteristic appearance of MCT. Special stains for amyloid should be done since the presence of this substance in a thyroid neoplasm is practically diagnostic of MCT.

Obviously, all four parathyroid glands should be located and evaluated at the time of the total thyroidectomy. The usual policy is to remove only grossly enlarged parathyroid glands. Should all four glands be enlarged, the treatment of choice is total parathyroidectomy with concomitant heterotopic parathyroid autotransplantation (27).

If biochemical and pharmacological testing indicates the presence of a pheochromocytoma, then the abdomen should be explored prior to exploration of the neck. When pheochromocytomas occur in patients with MEN-II, they can be expected to be bilateral in 70% of cases.

## RESULTS OF TREATMENT

Biologically, MCT is thought to be a more aggressive neoplasm than papillary or perhaps follicular carcinoma of the thyroid gland, but much less aggressive than anaplastic thyroid carcinoma. The reported 10 year survival has been approximately 60–70% (28) and depends on the presence of lymph node involvement at the time of the initial tumor resection. With utilization of provocative testing in family members at risk, the diagnosis should be made early and treatment instituted before the disease spreads beyond the thyroid gland. Accordingly, recurrence intervals and survival rates should be improved, although sufficient patients have not been evaluated for a long enough time to establish this point. Having completed the primary operative treatment for MCT, patients should be screened at six-month intervals by provocative testing to detect elevated TCT levels. Should the disease recur, appropriate therapy can be instituted.

Medullary carcinoma of the thyroid seems to be relatively radioresistant, and has been shown not to concentrate radioactive iodine (29). Recently Gottlieb & Hill (30) have reported a response to chemotherapy with Adriamycin® in three of five patients with MCT. The usefulness of this and other chemotherapeutic agents in the treatment of metastatic MCT, however, remains to be clarified.

## SUMMARY

The clinical characteristics, methods of diagnosis, and treatment of MEN-II have been described. Attention in the future should be focused on diagnosing MCT at the earliest possible time in MEN-II family members. The development of chemotherapeutic or other antitumor agents for the treatment of metastatic MCT should prove a fertile field for investigation.

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# NEW OBSERVATIONS IN CROHN'S DISEASE[1]

❖7198

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## INTRODUCTION

Ten years ago in this publication (1) the senior author and his colleagues ambitiously entitled their review "Granulomatous diseases of the gastrointestinal tract." The present one is more modest in scope; it attempts to bring together some "New observations in Crohn's disease."

To avoid metaphysical hairsplitting and semantic bogs, we define Crohn's disease as a chronic inflammatory disorder of the intestinal tract of unknown origin that may appear at any site from mouth to anus, but that characteristically involves the distal small bowel and colon, usually sparing the rectum. Clinically it is a diarrheal disease with relatively infrequent bleeding, associated with fever, abdominal masses, anal and perianal lesions, and growth retardation in the young. Enteric fistulas and sinus tracts are characteristic. The involved gut displays linear ulcerations, deep fissures, and enlarged regional lymph nodes. Histologically, the inflammation may spare the mucosa but involves all other layers, especially the submucosa. It is characterized by the aggregation of chronic inflammatory cells, especially histiocytes and macrophages, and by the presence of sarcoid-like non-caseating granulomas, which may extend throughout the entire thickness of the bowel wall and be present in the regional lymph nodes.

We assume that Crohn's disease is one disease wherever it occurs, and urge that it should be separated from other "nonspecific" inflammatory diseases of the bowel, although we grant that this may not be possible in all cases.

## SOME CLINICAL ASPECTS

### *Distribution of Disease*

Despite the epidemic of publications on Crohn's disease of the colon since the early 1960s, limitation to the colon remains the rarest form of the disease. In an unpub-

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[1] Aided by a grant from the National Foundation for Ileitis and Colitis, Inc.

lished study from this institution, Greenstein observed that among 700 patients with inflammatory bowel disease (IBD) seen in the decade 1964–1973, 202 were patients with ulcerative colitis; of the remainder, regional enteritis (mainly ileitis) comprised 213; Crohn's ileocolitis, 223; and Crohn's disease of the colon alone, 62. A similar distribution of Crohn's disease in subjects developing the disease before age 21 has recently been reported from the Mayo Clinic (2).

Difficulties in assigning a specific patient with IBD to either Crohn's disease or ulcerative colitis occur in perhaps 10–15% of any large series of patients, even when the best of current clinical, radiographic, and pathological criteria are pooled. This problem arises mainly when the colon alone is involved, the rarest form. While some would interpret this difficulty as evidence of the unitary nature of IBD with a wide spectrum of manifestations, we feel it should rather encourage the search for other bowel diseases, since the colon has a limited repertoire of responses. The recent differentiation of ischemic colitis has added a third entity. There are probably many more entities to be disentangled.

### *Problem of Cancer*

The recorded association of over 20 cancers of the ileum with Crohn's disease (3) must be accepted as being beyond the realm of chance, in light of the extreme rarity of adenocarcinoma in this location. In view of the documented alarmingly high risk of colon cancer in ulcerative colitis, the recognition of Crohn's disease of the colon clearly raises this important problem anew. The evidence is just beginning to be assembled beyond isolated case reports; the Mayo Clinic study cited above (2) suggests that in the younger age group (as in ulcerative colitis), the risk of cancer is increased, but nowhere with the dimension of the ulcerative colitis association. Unfortunately, measurement of the carcinoembryonic antigen does not help in Crohn's disease, since in a study from this institution, 40% of Crohn's disease patients had elevated values without evidence of cancer (4).

### *Extraintestinal Manifestations*

The association of IBD with a wide variety of extraintestinal complications remains a fascinating clinical problem. In Crohn's disease it is useful to group together those manifestations that appear to be related to the "colitic" activity of the disease, and to differentiate them from those associated with disturbances in the pathophysiology of the small bowel.

"COLITIS"—ASSOCIATED MANIFESTATIONS Manifestations associated with colitis involve the mouth (aphthous stomatitis), skin (erythema nodosum and pyoderma gangrenosum), eyes (episcleritis and uveitis), liver (fatty infiltration and pericholangitis, and rarely cirrhosis, sclerosing cholangitis, and even bile duct carcinoma, with granulomatous hepatitis most unusual), and peripheral joints (arthralgias and arthritis). Ankylosing spondylitis and sacroiliitis require separate discussion.

In the series of 498 patients with Crohn's disease collected by Greenstein at Mount Sinai Hospital (1964–1973), this group of manifestations occurred in 24%

of the ileitis, 39% of the ileocolitis, and 55% of the Crohn's colitis patients (compared to 48% of 202 patients with ulcerative colitis). They usually appear to be related to the severity of the colonic activity of the disease, to fluctuate in intensity with the underlying bowel disease, and to respond to the treatment of the bowel disease. Arguing from analogy with the arthritis of hepatitis, the arthritis of intestinal bypass operations for obesity, and some forms of glomerulonephritis, current thinking tends to view this group of extraintestinal manifestations as phenomena related to the deposition of immune (antigen-antibody) complexes in extraintestinal tissues (5).

ANKYLOSING SPONDYLITIS Ankylosing spondylitis is of much interest in the context of Crohn's disease, since the spondylitis has a clear-cut genetic pattern of inheritance in contrast to Crohn's disease, yet occurs in Crohn's up to 20 times more commonly than in the general population. In some series (6, 7), between 3 and 12.6% of Crohn's patients have ankylosing spondylitis, and in another (8), sacroiliitis has been recorded in 18% of 116 patients. The genetic implications of this association with spondylitis are discussed in the section on epidemiology. In contrast to the complications of the "colitis"-related group, these joint manifestations are not clearly related to the extent or severity of the bowel disease. In contrast also to the peripheral arthritis of the colitis group, ankylosing spondylitis may begin years before the bowel disease. The usual male preponderance in ankylosing spondylitis is said not to hold for patients with IBD.

SYNDROMES RELATED TO SMALL-BOWEL PATHOPHYSIOLOGY. A smaller but no less interesting group of syndromes is clearly related to disturbances in small intestinal (especially ileal) function, which plays a critical role in the absorption of vitamin $B_{12}$ and bile salts, and thus in triglyceride absorption, calcium and oxalate balance, and fluid and electrolyte absorption. In the series of 498 patients with Crohn's disease from this institution (cited above), between 3 and 13% of the patients had syndromes related to these malabsorptions. In general they were not related to the severity or activity of the disease, but rather to the anatomical extent of the ileal involvement.

At the Mount Sinai Hospital, in 65 patients with Crohn's disease of the small intestine there was a close correlation between length of diseased or resected ileum and vitamin $B_{12}$ and fat malabsorption (9). When the length of ileal dysfunction exceeded 90 cm, the Schilling test was abnormal in all 13 patients studied, and steatorrhea became more marked. Patients with resection and postoperative recurrence of disease had significantly less fat and $B_{12}$ absorption than those who had not been operated upon. Jejunal function as measured by D-xylose absorption was generally preserved in most of the patients studied.

## *Gallstones in Crohn's Disease*

While earlier clinicians were aware of an apparent increase in gallstones in Crohn's disease of the ileum, it is only recently that statistical evidence of this increase has begun to be documented. Heaton & Read (10) found stones in 23 of 72 patients

(31%) with disturbed ileal function and bile salt metabolism, the majority with Crohn's disease; Cohen et al (11) noted them in 14 out of 41 patients studied (34%). The recent explosion of investigations into the ileal absorption of bile salts has established a firm pathophysiological basis for this complication. Ileal dysfunction or resection leads to a diminished bile salt pool and thus to a reduction in the bile salt/cholesterol ratio in the gallbladder, which in turn favors cholesterol precipitation. In the Mount Sinai study of 498 patients with Crohn's disease, a little more than 10% were recorded as having gallstones. Although no systematic study of this was made, analysis does indicate that involvement of the ileum appears to be the critical factor: 49 patients had stones (12%) when the ileum was involved, only 3 (5%) when it was not.

## *Renal Complications*

OBSTRUCTIVE HYDRONEPHROSIS A study from this group in 1969 (12) called attention to the development of obstructive hydronephrosis and hydroureter in Crohn's disease as the result of extension of the inflammatory process into the retroperitoneal areas from a localized perforation, usually right-sided with ileal disease, but occasionally left-sided or even bilateral with Crohn's disease of the colon. Pain in the hip or thigh, or a limp may be important diagnostic clues. It is important to treat the bowel disease, not the kidney. While resection was required in the majority, we have seen relief of the obstruction in a few patients responding to medical treatment.

KIDNEY STONES The development of kidney stones represents an important and fairly frequent complication of Crohn's disease, ranging up to 10% in some large series. We have observed an incidence of 8.5%, mostly in patients with ileal resection or ileostomy.

Low urinary volume and increased solute concentration caused by impaired intestinal reabsorption of water, together with low urinary pH secondary to intestinal loss of $HCO_3$, contribute to the formation of uric acid stones. We have also observed hyperuricemia in both male and female patients, possibly related to increased tissue breakdown.

The most interesting recent advance in understanding some aspects of nephrolithiasis in Crohn's disease has been the confirmed observation that some patients, especially those with ileal resection, have oxalate stones as the consequence of hyperoxaluria, which arises not from any renal defect in the handling of oxalate, but from increased intestinal absorption of oxalate (13, 14). Recent studies demonstrate that steatorrhea, with its intestinal loss of calcium, is associated with a reciprocal increase in oxalate absorption. Therefore, measures designed to avoid high-oxalate foods (spinach, tomatoes, rhubarb) and to reduce the steatorrhea (low-fat diet or supplements with medium-chain triglycerides) appear to be useful (15). Methods for binding oxalate in the intestinal tract are now under investigation.

RENAL AMYLOID Despite contrary impressions, renal amyloid occurs more frequently in Crohn's disease than in ulcerative colitis. In our series, 5 of 468 patients with Crohn's disease had clinically significant renal amyloid, while 202 patients with ulcerative colitis had none. The lesion can now be identified by rectal or renal biopsy. A recent case report documents regression of both hepatic and renal amyloid following resection of the Crohn's disease (16).

## THERAPEUTIC CONSIDERATIONS

The natural history of Crohn's disease since its first description in 1932 remains unknown. No large, long-term, untreated series of patients has ever been reported. The placebo group in the current National Cooperative Crohn's Disease Study from a large number of university centers may yield data on the course of the disease in patients receiving only a placebo for periods of 18 and 24 months.

Currently, aside from symptomatic medication, most patients who are sick (or in some cases even quiescent) are receiving one of three drugs alone or in combination: corticosteroids (mainly prednisone), Azulfidine® (or rarely other sulfonamides, especially of the poorly absorbed oral variety), and immunosuppressive drugs (mainly azathioprine [Imuran®]). Few long-term, large, controlled studies of these drugs alone or in combination exist. One short-term study of Azulfidine carried out in 25 outpatients for 4 months revealed no significant difference between the drug and placebo (17). Azathioprine was introduced by B. N. Brooke and his colleagues in 1969 with great success in the closure of enterocutaneous fistulas in six patients (18). Of five controlled trials of azathioprine recorded to date, three (19–21) showed no significant usefulness for the drug in 53 patients, treated for periods of 2–24 months. In two other studies, involving 42 patients (10 of whom also received prednisone) treated for 6 months, improvement was reported in the treated group as judged by clinical assessment in one study (22) and by reduction in steroid dosage in the other (23).

Because of this unsatisfactory state of knowledge regarding these widely used medications, a large, multicentered, controlled clinical trial in Crohn's disease is now under way, sponsored by the National Institute of Arthritis, Metabolism and Digestive Diseases. Patients with active or quiescent disease receive one of four drugs: placebo, Azulfidine, prednisone, or Imuran for periods up to 24 months. The problem of Imuran toxicity, especially pancreatitis, has raised considerable anxiety. In any event, we look forward to the results of this complex, detailed trial with great interest.

Parenteral hyperalimentation, administering hyperosmolar solutions by central venous catheterization, is currently being employed in IBD, especially in patients with complicated Crohn's disease of the small bowel, partial intestinal obstruction, extensive mucosal involvement, or intestinal and perianal fistulas. While favorable results have been reported in terms of improvement in nutritional status and even closure of some fistulas (24, 25), controlled studies and large series with long-term follow-up have yet to be reported. We view this technique as supportive, or at times important as a preoperative adjunct.

In a disease as enigmatic as Crohn's, and as difficult to treat, it is understandable that variations of rational and irrational therapies are frequently proposed. Since some patients with Crohn's disease display manifestations of immune deficiency, attempts to stimulate the immune system have been advocated. A few cases of ileitis have been treated with Bacillus Calmette-Guérin (BCG) by intradermal injection (26), and a controlled trial with orally-administered BCG is now under way.

Aside from those patients with Crohn's disease operated upon for surgical emergencies (intestinal obstruction, perforation, and massive hemorrhage), all others who come to surgery do so on account of the inadequacy of our present-day medical measures. It is generally held that three fourths of patients with Crohn's disease do come to operation, although data on this point are sorely lacking. In our study of some 160 patients with Crohn's disease, 100 or 62.5% came to surgery (50% of the 30 with "pure" colitis, and 65% of the ileocolitis group) (27).

Many problems surround the evaluation of the results of surgical treatment of Crohn's disease, based in part on the variety of operations used, differing surgical indications, inadequate patient matching, and especially failure to define and search for recurrences systematically. We have recently tabulated the literature on crude recurrence rates following the major types of surgical interventions in the major categories of Crohn's disease (28). The wide scatter of these data makes it hard to believe that one disease could behave so differently in different surgeons' hands. Further, considerable debate has centered around the rate of recurrence of Crohn's disease in the ileum following excisional therapy of Crohn's disease of the colon. The high recurrence rates we have observed (29) have recently been confirmed by studies from Trevor Cooke's group in Birmingham, England (30). The apparent confusion in these data is clarified, however, when instead of crude recurrence rate, modern actuarial (life table) techniques are employed. If this is done, then the inexorable tendency of Crohn's disease to recur is clearly demonstrated (27). In the only available study of Crohn's disease of the ileum alone, treated by resection and analyzed in terms of cumulative recurrence rates of the population at risk, Lennard-Jones & Stadler (31) reported symptomatic recurrences at 5 years in 31% of the patients, and in almost 60% of those followed for 10 years.

Goligher and his associates at Leeds (32) have presented their data at a recent symposium, lumping patients into two large groups—80 mainly small-bowel cases (17 treated by small-bowel resection and 63 by straight hemicolectomy with more or less small-bowel resection) and 90 mainly large-bowel cases (composed of 33 treated by right hemicolectomy alone, 37 proctocolectomy without significant small-bowel resection, and 20 by varieties of partial or subtotal colectomy). This group noted two phases of recurrence, an early one within the first 2 or 3 years after operation and a second following 3 or 4 years of quiescence. While the cumulative recurrence rates for colonic disease were lower than those for the small-bowel disease, both were substantial; at 10 years the former was 33% and the latter 62.5%. In our series of Crohn's disease of the colon and ileocolitis, the cumulative reoperation rate (surely a good criterion of clinically significant recurrence) reached 89% by the fifteenth year (27). This was true of patients treated by either excision or bypass, and in those whose illness started either before age 25 or later.

In light of the above data, therefore, it may be doubted that physicians or surgeons are significantly improving the long-term course of their patients with this enigmatic disease.

## EPIDEMIOLOGY

The two primary goals of epidemiologic research in Crohn's disease are, first, to learn how many patients are afflicted with the various forms of this disorder, and second, to determine what the factors are that place people at risk. Without knowledge of an etiologic agent, and without consistent and universally recognized criteria of diagnosis, the problems of case finding, classification, and recording are formidable. Nonetheless, enough encouraging progress has been made in the past ten years to permit at least some preliminary answers.

### *Incidence and Prevalence*

In the middle 1960s, a number of studies from the United States, the United Kingdom, and Scandinavia suggested that the annual incidence of Crohn's disease was less than 1–2 new cases per 100,000 population (33–36). Many of these data, however, tended to underestimate the true incidence for any of several reasons: omission of cases involving the colon, exclusion of cases in teenagers and children, and tabulation of hospitalized cases only. Most newer studies throughout this past decade have indicated that the annual incidence is actually in a higher range, approximately 2–4 per 100,000 population, with a prevalence in the area of 20–40 per 100,000 (37–41). Incidence and prevalence figures are heavily influenced, though, by methods of case finding, so that a mail survey of hospitals yields a relatively low result of 1.0 per 100,000 incidence (42), while a thorough search of a sophisticated record-linkage system produces an annual incidence figure of 6.1 per 100,000 and a prevalence of 60 per 100,000 population (43).

### *Risk Factors*

Crohn's disease is not an ailment predominantly of either males or females, but it is chiefly an affliction of younger people, with peak incidence of onset occurring in teenagers; indeed, nearly 60% of all cases begin between ages 10–25 (44). A second smaller age-incidence peak is often observed during the sixth decade of life, and comprises mostly colitis cases that tend to follow a different clinical pattern, and may represent a somewhat different pathophysiologic entity from classical regional enteritis (33, 44). There has not consistently been observed any significant predilection of the disease for urban versus rural populations nor for upper versus lower socioeconomic groups, but striking ethnic variations have been noted. Although the Swedish (38, 39) and Scottish (34, 37) series contained no Jewish patients, most American series have demonstrated a distinct four- to six-fold proclivity among Jews (45–47). By contrast, the incidence is proportionately much lower than expected among black people (36, 48), American Indians (48), and Spanish-Americans (49).

There is also a definite, though by no means simple, tendency for Crohn's disease to run in families. Many cases of ileitis in twins and among multiple family members have been recorded and thoroughly reviewed (50, 51). Prior to the previous review of granulomatous bowel disease in this journal ten years ago (1), the most extensive original study of familial aspects of Crohn's disease (and ulcerative colitis) had come from Kirsner's group in Chicago (52). Within this past decade, the foremost study of familial patterns has again come from the same department (53), with a report of at least 113 of 876 patients (12.9%) with IBD having positive family histories; a randomly selected group of 150 patients from this series had an 11% incidence of positive family histories, compared to only 4% of carefully matched normal control subjects. This familial tendency was most pronounced among younger patients and among Jews.

Recent studies of genetic aspects of IBD have spotlighted three features of particular pathogenetic interest. The first point is that positive family histories of IBD seem to occur more frequently among patients with Crohn's disease than among patients with ulcerative colitis, and that patients with Crohn's disease have relatives with ulcerative colitis more frequently than patients with ulcerative colitis have relatives with Crohn's disease (52). While the latter observation could simply be a reflection of the fact that ulcerative colitis is a more common disease than regional enteritis, these facts have led McConnell to formulate a hypothesis that the two diseases could have a quantitatively related genetic basis, with the more complete genotype predisposing to Crohn's disease and the less complete genotype predisposing to ulcerative colitis (50, 54). This hypothesis might also help explain why affected relatives of patients with Crohn's disease have Crohn's disease so much more often than do affected relatives of ulcerative colitis patients (56% vs 16%) (52).

A second important genetic observation of recent years is that the histocompatibility antigen (HL-A) W27, which occurs with high frequency in idiopathic ankylosing spondylitis and in uveitis, is also unusually common in IBD patients with spondylitis (55–58). Sacroiliitis and spondylitis associated with IBD tend very strongly to cluster with uveitis in the same patients (55, 59, 60); spondylitis and uveitis are both strongly associated with the W27 antigen in patients with (55–58) and without (61–64) IBD; spondylitis and uveitis both tend to run clinical courses independent of the status of their associated bowel disease (8, 65–67); and even unaffected relatives of IBD patients have a higher than expected frequency of spondylitis (68, 69)—all of these data strongly suggest that predispositions to IBD, ankylosing spondylitis, uveitis, and HL-A antigen W27 all have overlapping genotypes.

The third major new genetic finding has been that clinically unaffected relatives of patients with ulcerative colitis have an increased prevalence of high-antibody titers against colon and enterobacterial antigens (70). This phenomenon could be attributable either to genetic or environmental factors, but it does tend to militate against a primary pathogenetic role for circulating antibody in the development of IBD, as is discussed further in the section on etiology and pathogenesis.

## PATHOLOGY

The past ten years have seen new developments in the gross anatomy, microscopic pathology, and biochemistry of Crohn's disease.

### *Gross Anatomy*

Crohn's disease has long been recognized as extending beyond the ileum to involve and sometimes even to primarily affect the jejunum, stomach, duodenum, colon, anus, and rectum. More recently, recognition has been given to cases affecting the mouth (71–73), esophagus (74–79), appendix (80, 81), and even the skin (82). Positive documentation of esophageal involvement, however, still awaits a case report of classical granulomatous pathology in the esophagus in a patient with evidence of Crohn's disease elsewhere in the gastrointestinal tract. Particular diagnostic features that identify granulomatous colitis either mimicking or coexisting with diverticulitis have been described by groups with special expertise in the pathology and radiology of Crohn's disease (83, 84).

### *Microscopic Pathology*

Since the most distinctive characteristic lesion of regional enteritis is the epithelioid, or sarcoid-type granuloma, it is inevitable that pathogenetic parallels should have been sought between ileitis and sarcoidosis. This is especially true since the two diseases also seem to share certain immunologic alterations and peripheral complications. While Mitchell and others have reported an unexpectedly high incidence of Kveim positivity among patients with Crohn's disease (85, 86), other groups of investigators have failed either to reproduce this phenomenon (87–90) or to find other serologic parallels between the two diseases (91).

Recent light microscopic studies of rectal biopsies taken from patients with quiescent colitis have suggested a significantly higher proportion of macrophages in the lamina propria in Crohn's disease than in ulcerative colitis (92). Electron microscopy has also implicated the lymphocytes and other mononuclear cells as possibly playing a critical role in the initial pathological lesion (93). This same ultrastructural study of Crohn's disease has revealed clusters of bacteria in the deep layers of the colon, not found in comparable specimens of normal or ulcerative colitis colon (93). These observations may someday prove to have significant implications for the pathogenesis of the disease process.

### *Biochemistry*

Until recently, the search for biochemical serum markers of Crohn's disease had been unrewarding, but within the past year it was reported that serum lysozyme levels were strikingly and specifically elevated in patients with granulomatous ileitis and colitis as opposed to normal controls or to most patients with ulcerative colitis (94). Additional studies by the same group have further suggested a close correlation between the activity of the disease and the magnitude of the lysozyme elevation (95), although this observation has yet to be confirmed by other investigators. In any event, since lysozyme is a recognized indicator of granulocyte and particularly of macrophage turnover in other granulomatous disorders, its elevation in Crohn's disease may not offer revolutionary new insights into pathogenesis, but may well turn out to be a useful and convenient adjunct in differential diagnosis and longitudinal follow-up.

## ETIOLOGY AND PATHOGENESIS

In recent years, the main thrusts of basic research in IBD have been directed into three interrelated areas: (*a*) autoimmunity, (*b*) cellular immune deficiency, and (*c*) transmissible infection.

### *Autoimmunity*

In their constitutional manifestations and their responses to corticosteroid therapy, inflammatory bowel diseases resemble other conditions widely regarded as "autoimmune" in origin. Such nonspecific observations, however, provide only tenuous and circumstantial evidence for the autoimmune etiology of IBD.

More provocative is the finding, repeatedly confirmed over the past 15 years, that there are circulating anti-intestinal antibodies in both ulcerative and granulomatous bowel disease, and that these antibodies cross-react with antigens from certain enteric bacteria such as *E. coli* 014 (96–99). Studies of these circulating antibodies and of other immunoglobulins have demonstrated that they can bind to intestinal epithelial cells (100), that they are apparently sequestered by affected intestine (101), and that their serum levels are elevated following resection of the diseased tissue (102). The pathogenetic significance of these findings, however, remains very much in doubt, since circulating antibodies may appear merely as a secondary manifestation of nonspecific tissue injury (103, 104), serum antibodies against intestinal tissue have not been shown to possess any cytotoxic potential (105), these antibodies can be demonstrated in some clinically healthy subjects (70, 106), and typical IBD has been observed to develop in patients with agammaglobulinemia (107–108).

Even more suggestive of an autoimmune pathogenesis of IBD is the observation that lymphocytes from patients with ulcerative and granulomatous colitis are cytotoxic to intestinal epithelial cells in vivo (109) and in vitro (110–113). The prolific studies of Shorter and his associates have further demonstrated that this property of anticolon cytotoxicity may be mediated by lymphotoxin (114, 115) and can be conferred upon normal lymphocytes and induced in colitis lymphocytes by preincubation either with crude lipopolysaccharide antigen from *E. coli* 0119:B14 or with the serum IgM fraction from patients with ulcerative or granulomatous colitis (116–119). These findings, together with the circulating antibody data described above, have prompted the hypothesis that IBD is the consequence of an unwelcome lymphocyte-mediated hypersensitivity reaction to bacterial antigens normally present in the lower gastrointestinal tract (120).

Indirect support for such a hypothesis is forthcoming from numerous studies during the past ten years that have demonstrated lymphocyte stimulation by various *E. coli* and intestinal epithelial and meconium antigens (121–129). Persuasive evidence is still lacking, however, either for the specificity of these antigens in one or another form of IBD or for their role in disease pathogenesis.

### *Cellular Immune Deficiency*

A sustained and increasing interest in cellular immune mechanisms in IBD has provoked a spate of studies concerning skin reactivity and in vitro lymphocyte responsiveness in Crohn's disease and ulcerative colitis (121–143). As might be expected from studies on different populations of patients, utilizing widely varying technological methods, the conclusions have been controversial. Our own opinion is that there probably are deficiencies in cell-mediated immunity among many patients both with Crohn's disease and with ulcerative colitis. This belief is supported by our findings of impaired lymphocyte responsiveness to the nonspecific mitogen phytohemagglutinin in one third of patients with both disorders (144); our observations and others' of low T-lymphocyte counts as contrasted with elevated B-lymphocytes in both diseases (145–147); and our finding of absent delayed hypersensitivity to skin testing with dinitrochlorobenzene among many patients in both groups (146). This phenomenon of defective cellular immunity in IBD could be relevant to pathogenesis, but the undeniable possibility also remains that all of these observed immunologic aberrations in IBD may simply represent secondary responses to some as yet undetermined primary etiologic agent.

### *Transmissible Infection*

Nearly 45 years of microbiologic and serologic studies have so far failed to implicate any specific microorganism as an etiologic agent in Crohn's disease. Abnormal bacterial flora are not observed in the small bowels of patients with regional enteritis except in the presence of contaminating complications such as stasis, fistulization, or ileocolic anastomosis (148). The newly recognized bacterium *Yersinia entercolitica* has been isolated from cases of "acute terminal ileitis," but these patients do not go on to develop subsequent Crohn's disease (149).

A new chapter in microbiological research in regional enteritis was opened with the 1970 report of Mitchell and Rees that classical epithelioid granulomas had been transmitted into mouse footpads by inoculation with ileal or mesenteric lymph node homogenates from a patient with Crohn's disease (150). Although this phenomenon was not universally reproducible (151), further studies by Mitchell and his associates (152, 153) and by ourselves (154, 155) have amply confirmed that homogenates of intestinal tissues from Crohn's disease patients do indeed transmit granulomatous lesions into mouse footpads (154, 155) and rabbit ileum (152, 153). The specificity of this transmissible agent and its physicochemical and biological characteristics have yet to be established (156). This avenue of investigation, however, seems to be among the most promising on the scene today.

## AN AFTERWORD

As we consider the emergence of Crohn's disease as a clinical and pathologic entity, we are struck by the paucity of recognizable cases of this disorder before the turn of the century. It strains the imagination to conceive how the existence of this dramatic disease could have been ignored or overlooked by the meticulous pathologists of the nineteenth century. More likely, it seems to us, the cases were simply not there. We appear to be witnessing the development and evolution of an entirely new disease.

If this is the case, then two corollaries follow. First, we should not castigate ourselves too harshly for our failure so far to find the cause and cure of Crohn's disease. After all, leprosy and syphilis existed for many centuries before their mysteries were solved, and Crohn's disease has been recognized for only 44 years. Second, we cannot yet know how this new disease is going to evolve, in terms of either its prevalence or its clinical manifestations. As a recent editorial has observed (157) we may be seeing what is only the beginning of a devastating epidemic.

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# SIGNIFICANCE OF SERUM PROTEIN AND TISSUE BINDING OF ANTIMICROBIAL AGENTS

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## INTRODUCTION

The prime objective of antimicrobial chemotherapy is to aid in eradicating invading microorganisms by delivering an optimal amount of active drug to the site of infection. The ability of a drug to reach effective concentrations at the site of infection depends on many physiochemical and pharmacologic characteristics. The binding of antimicrobial agents to macromolecules is one of the important determinants. Antimicrobial agents are now known to bind variously to constituents of blood, cell membranes, and intracellular proteins. This report reviews the effect of different forms of binding on the pharmacokinetics, biological activity, and potential toxicity of antimicrobial agents. Four principal types of binding will be considered: (*a*) serum proteins, principally albumin; (*b*) cell membranes; (*c*) soluble intracellular proteins; and (*d*) insoluble intracellular substances.

## SERUM PROTEIN BINDING

Antimicrobial agents that combine with serum proteins do so by forming a reversible bond with serum albumin. Antimicrobials are diverse chemicals and must be considered individually. Small variations in charge or chemical configuration can exert profound changes in binding, so that generalizations should not be made, even within a class of drugs. The marked variation in binding observed with the penicillins (1, 2) becomes clear when one realizes that it is the aromatic side chain of the molecule that is primarily involved in binding of these drugs to serum albumin (3).

The extent of binding is affected by concentration of drug and albumin, by pH, and by the presence of compounds that might be bound to the same or adjacent sites on the albumin molecule (2, 4). It is therefore important that experimental conditions utilized to measure protein binding simulate the in vivo situation. For example, binding of gentamicin to serum proteins under normal physiologic conditions is minimal. Binding is markedly increased if the method artificially reduces the concentration of divalent cations (5).

Serum protein binding becomes an important consideration when it exceeds 70–80%. This is because small changes in binding have the greatest effect on the proportion of free drug as the fraction of bound drug increases. For example, a reduction of binding from 98% to 96% will double the amount of free drug available, whereas a drug that is 60% bound would have to have its binding reduced to 20% to do the same. Within certain classes of antimicrobials, such as the penicillins and tetracyclines, there is a good correlation between the degree of protein binding and lipid solubility (6–8). For example, the more highly protein-bound penicillinase-resistant penicillins, such as nafcillin, oxacillin, cloxacillin, and dicloxacillin, are much more lipid soluble than the slightly or moderately bound analogues such as methicillin, ampicillin, and penicillin G. Similarly, minocycline and doxycycline are more lipid soluble and more highly bound by serum albumin than is tetracycline.

Extensive serum protein binding of antimicrobial agents has several major effects, including restricted tissue distribution, reduced penetration into interstitial spaces and inflammatory fluids, delayed elimination, and interference with biologic activity. Each of these effects is relatively easy to understand in pharmacologic terms. Unfortunately, they have often been misinterpreted in the literature or misrepresented in advertisements. It is therefore important to characterize each effect and interpret the evidence currently available.

### *Effect on Tissue Distribution*

Serum protein binding appears to be one of the important determinants of distribution of drugs in the body. It is only the free, unbound drug that can readily pass through the capillary pores into tissue fluids (9). Highly bound drugs tend to remain in the intravascular compartment, giving high blood levels, while slightly bound drugs diffuse more rapidly into the interstitial fluid. Other factors such as tissue binding, lipid solubility of the drug, and its degree of ionization at pH values in blood and the tissue fluids also influence tissue distribution and can modulate the effect of binding to serum proteins. Pharmacokinetic models have shown that serum protein binding has less effect on a drug that is able to concentrate in tissues than on one that is restricted to the extracellular fluid (10).

The influence of serum protein binding on in vivo distribution of antimicrobial agents has been best studied with the penicillins and sulfonamides. These agents distribute primarily in the extracellular space. Kunin (11) has shown that the distribution of three penicillin analogues in rabbit tissues was inversely related to their binding in rabbit serum. He further demonstrated that infusion of *ortho*-cresotinic acid, shown in vitro to displace ancillin from protein, increased distribution of this penicillin derivative in rabbit tissue. Anton (12) has shown similar results in animals given sulfonamides and agents that compete for sulfonamide binding sites.

Additional evidence is available with the penicillins in man (13). Administration of sulfamethoxypyridazine prior to an injection of oxacillin reduced the serum protein binding of oxacillin, resulting in a doubling of free drug available in serum.

This was accompanied by a fall in total oxacillin in serum, presumably due to enhanced distribution of unbound drug. Despite the lower levels of total drug, more was available in the serum in the free unbound form.

An experiment of nature exists in which the serum of uremic patients binds certain drugs to a lesser extent than does normal serum (14). This binding defect has been primarily observed with weak organic acids (15) and therefore applies to many antimicrobials, such as the penicillins, cephalosporins, and sulfonamides (16, 17). Craig and associates (18) have presented strong evidence that the defect in binding in uremia is due to accumulation of a binding inhibitor. Single-dose pharmacokinetic studies with cefazolin and sulfamethoxazole (19, 20) have demonstrated that this binding defect tends to lower total blood levels and raise the volume of distribution, because free drug can diffuse more readily from the intravascular compartment. Similar pharmacokinetic effects occur in the nephrotic syndrome and other hypoalbuminemic states (21).

### *Effect on Penetration into Interstitial Space and Inflammatory Fluids*

Gross recovery of a drug from tissue homogenates provides limited information about its disposition in the tissue. Binding of the drug to various tissue components and contamination with blood and the other body fluids can greatly affect tissue concentrations. Various models have therefore been utilized to study the effect of serum protein binding on penetration of drugs into interstitial fluid. Lymph, as it enters the peripheral lymphatics, appears to be representative of interstitial fluid (22). Verwey and Williams (23) conducted a study of canine lymph/plasma ratios with various penicillins under conditions of constant intravenous infusion. Concentrations achieved in lymph were inversely related to the proportion bound to plasma. Furthermore, the concentration of unbound drug in lymph approximated the unbound concentration in plasma (24).

Barza, Samuelson & Weinstein (25) studied the penetration of various antibiotics into fibrin clots implanted subcutaneously in rabbits. The concentrations in fibrin clots of ampicillin and methicillin, two weakly bound agents, were significantly higher than the more strongly bound oxacillin and nafcillin. In fact, concentrations were directly related to the concentration of free, rather than total, antibiotic in serum. McQueen (26) implanted dialysis bags into the peritoneum of rats and showed that the sulfonamide concentration in peritoneal fluid was similar to the unbound fraction of drug in plasma. Antibiotic concentrations in canine pericardial fluid also appear to be dependent on the percentage of antibiotic bound to serum albumin (27).

A method has also been described (28) to measure the appearance of antibiotics in human interstitial fluid. An area of skin was denuded and covered with a chamber filled with physiologic saline solution. Subjects were then given a variety of penicillins, and the appearance of drug into the chamber was compared at intervals to levels in the serum. Antibiotics with lower proportions bound to serum proteins diffused into the fluid to a greater extent than antibiotics that were highly bound. Flucloxacillin, 95% of which is bound to protein, gave no detectable levels in the skin window.

Penetration of ampicillin and cloxacillin into synovial fluid of patients with osteoarthritis and rheumatoid arthritis is dependent upon serum binding of these drugs (29). Ampicillin, a lightly bound drug, appeared in synovial fluid at the same concentrations as in serum, whereas cloxacillin levels in joint fluid were much lower than in serum. The important finding, however, was that unbound levels of cloxacillin in synovial fluid and serum were similar, thus indicating that the distribution was primarily dependent on the fraction of free drug.

### *Effect on Drug Elimination*

The effect of serum protein binding on glomerular filtration of drugs is well known. Among the antimicrobial agents, this is best exemplified by several cephalosporin analogues (30). Cephaloridine, cefazolin, and cephanone are all primarily eliminated from the body by glomerular filtration. As the serum protein binding of these analogues increases, the serum half-life increases. A similar relationship has also been shown with the tetracyclines and sulfonamides (31, 32).

In contrast to glomerular filtration, tubular secretion is apparently not limited by serum protein binding. This is clearly evident with the renal elimination of the penicillins (33). For example, methicillin, oxacillin, and cloxacillin all show similar rates of elimination even though they differ markedly in the extent of binding to serum proteins.

The effect of serum protein binding on hepatic metabolism and biliary excretion is less clear. Binding to serum proteins may hasten elimination of antimicrobial agents by the liver by increasing their rate of removal from other tissues and carrying them to the liver. A more probable effect of serum binding is a retardation of the elimination process. The uptake by rat liver slices of azidocillin, a highly bound penicillin analogue, was significantly reduced in serum, whereas the uptake of ampicillin was unaffected (34). Similarly, Newbould & Kilpatrick (35) found that reduction by plasma of the rate of sulfonamide acetylation in the perfused rabbit liver was directly related to the degree of protein binding. Their results suggest that only free sulfonamide is available to the hepatocyte.

### *Effect on Biologic Activity*

The effect of serum on the in vitro activity of a wide variety of antimicrobial agents has been extensively studied by many investigators (1, 2, 36–38). In each instance, a close correlation has been demonstrated between the amount of free drug and biologic activity. Kunin (39) was able to calculate binding from minimal inhibitory concentrations in broth and serum using arithmetic linear dilutions. With a sensitive photometric assay of growth inhibition of *Staphylococcus aureus,* Joos & Hall (40) were likewise able to calculate binding from growth inhibition of penicillins in the presence of albumin. Both investigators demonstrated that bound drug was unavailable for antibacterial activity. Furthermore, the bacterial inoculum did not exert a discernible effect in removing the drug. Thus, only free drug in serum is available for antimicrobial activity.

Biologic activity in vivo is related to additional factors besides the concentration of free drug in serum. These factors include (*a*) the intrinsic antimicrobial activity of the drug being administered, (*b*) the organ involved by infection, and (*c*) special effects of the antimicrobial on microorganisms or the host.

INTRINSIC ANTIMICROBIAL ACTIVITY The intrinsic activity of an antimicrobial is clearly important in interpreting serum concentrations of various penicillins. When administered in equal amounts, methicillin produces higher concentrations of free drug in serum than dicloxacillin (1). In general, the minimum inhibitory concentration (MIC) of methicillin against staphylococci is 1.5–3.5 μg/ml, whereas dicloxicillin is active at levels of about 0.1 μg/ml. When both binding and MIC values are taken into consideration, the biologic activity of both drugs in serum is about the same.

It is frequently assumed that increasing concentrations of drug above the therapeutic level would be associated with a greater biologic effect. Studies in the test tube (41, 42), animals (43), and man (44) have clearly demonstrated that this is not the case with the penicillins. Similarly, bacteriostatic agents remain bacteriostatic at the concentrations usually achieved in serum and tissues. In contrast, increasing concentrations of the aminoglycosides are associated with an increased rate of bacterial killing (45), but these agents have negligible binding to serum proteins (46).

SITE OF INFECTION There are several organs in the body in which tissue concentrations are not necessarily related to the concentration of free drug in serum. The kidney, gallbladder, and brain are the primary examples. In the kidney, drugs tend to be concentrated in the medullary region along with other solutes. This has therapeutic significance since infections of the renal parenchyma usually involve this same area. Urinary pH and the state of hydration have been shown to be important determinants of antimicrobial concentrations in the interstitium of the renal medulla (47, 48).

SPECIAL EFFECTS OF ANTIMICROBIALS Subinhibitory concentrations of nafcillin have been reported (49, 50) to increase the susceptibility of staphylococci to phagocytosis and lysis by lysozyme. Other penicillin analogues are ineffective in this regard. On the other hand, therapeutic concentrations of tetracycline have been demonstrated to depress phagocytosis and leukotaxis (51, 52).

### *Effects on In Vitro Sensitivity Tests*

Reduction in biologic activity by binding to serum proteins is important in evaluating the serum bactericidal test (53). The use of serum rather than broth for serial dilutions results in a significant decrease in the bactericidal titer for the highly bound penicillins. Values obtained with serum would be expected to more closely approximate the situation in the patient. Protein binding can also reduce the zone size obtained in microbiologic assays (54). It is important to utilize the same body fluid for the standard curve that is being tested for antibiotic concentration. A standard curve in fluids with a higher protein concentration than the unknown can result in falsely elevated concentrations. Likewise, a standard curve in fluids with a lower protein concentration than the unknown can give falsely low values.

## TISSUE BINDING

Antimicrobial agents are not only influenced by bindings to constituents of plasma, but also by cells. As shown in Figure 1, cellular uptake of drugs is dependent upon a series of dynamic processes including: (*a*) serum protein binding, (*b*) nonionic diffusion, (*c*) binding to cell membranes, (*d*) binding to intracellular proteins, and (*e*) biotransformation and release of metabolic products from cells. Each antimicrobial has unique properties that govern the extent to which these phenomena control its uptake and distribution within cells. Access to cells is dependent upon the proportion of free drug available within the bathing medium at a given point in time. Similarly, egress from cells will depend upon the intracellular concentration and the kinetics of binding to intracellular sites. The membrane may also bind drugs and greatly influence the exchange between the various compartments. These relationships are probably valid for exchanges between plasma and cells and for special regions, such as within the sinusoids of the liver, but may be quite different within areas such as the renal tubular and gastrointestinal lumina and in extravascular areas where serum protein binding effects are more limited or absent.

### *Penetration of Antimicrobial Agents into Cells*

It is well known that nonionic diffusion of substances across membranes is clearly related to their lipid solubility. This in turn depends, for weak acids and bases, on their $pK_a$ and the relative pH across a membrane barrier. Among the antimicrobials, highly lipid-soluble drugs include rifampin, trimethoprim, chloroquine, isoniazid, and chloramphenicol. These drugs are either neutral compounds or weak bases. They readily penetrate membranes, and for the most part passage is not limited by serum protein.

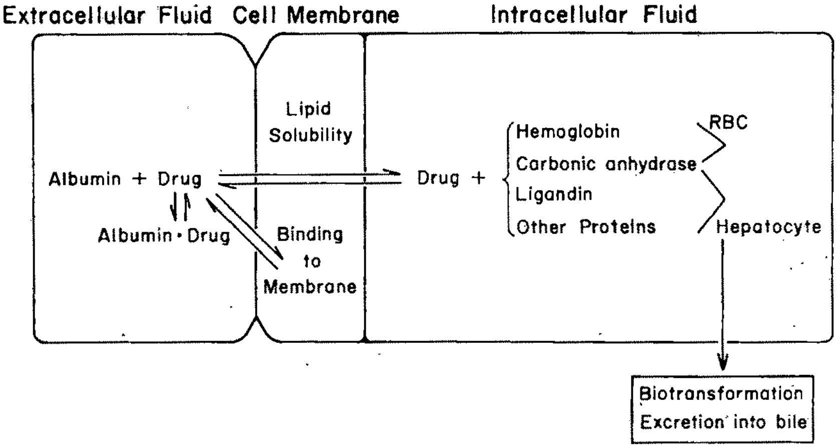

*Figure 1* Schematic representation of the effect of serum and intracellular binding proteins, lipid solubility, and membrane binding on distribution of drugs in intracellular and extracellular fluid.

The situation is more complex with weak acids such as the penicillins, cephalosporins, and tetracyclines. These agents are bound by serum proteins roughly in proportion to their lipid solubility (6). It therefore could be predicted that the more protein-bound analogues of the penicillin and tetracycline series would also more readily penetrate cells. This was shown by Kornguth & Kunin (55) in studies using human erythrocytes. Dicloxacillin enters human erythrocytes more rapidly and completely than penicillin G, and minocycline enters more rapidly than tetracycline when the cells are exposed to the drugs in the presence of nonprotein-containing buffer solutions. Uptake of the more lipid-soluble analogues, however, is markedly inhibited in the presence of whole serum or plasma. This is due to the more extensive protein binding of the more lipid-soluble compounds which limits the proportion of free drug available for diffusion into the cells. In addition, once the drugs have entered the cells, the more highly bound analogues are more readily released into serum-containing medium. The more lipid-soluble compounds are able to diffuse rapidly out of the cells and to be bound and sequestered by the proteins within plasma.

Lipid solubility thus exerts a pivotal role, both with respect to protein binding and to nonionic diffusion across membranes. This tends to balance the forces that govern the relationship between extracellular and intracellular concentrations of drug. Other forces, however, also influence this dynamic relationship. Among these are the effect of intracellular binding proteins, described below, which also bind anionic drugs and may force the equilibrium in the direction of the intracellular compartment.

The anionic antimicrobials appear to participate in what may be teleologically described as the protective mechanism of serum protein binding of potentially toxic lipid-soluble compounds. This mechanism solubilizes the agents within the blood and limits their passage into cells where they may do damage. The best example in which the effect may be important is with bilirubin, a highly lipid-soluble, highly protein-bound drug. Bratlid (56) has shown, for example, that the binding of bilirubin to erythrocytes is inhibited in the presence of albumin, but is increased when the bilirubin is displaced from the protein by highly bound antibiotics, sulfonamides, and free fatty acids. The analogy between bilirubin metabolism and anionic antibiotics is discussed more extensively below when we consider the phenomenon of intracellular binding proteins.

### *Binding to Cell Membrane*

The polymyxin antibiotics (polymyxin B and colistin) exert their antibacterial effect by binding to acid phospholipids within the cell wall and membranes of gram-negative bacteria (57). Similarly, the polyene antibiotics such as amphotericin B and nystatin bind to sterol-containing membranes of fungi (58). It is therefore not surprising that these drugs have been found to bind to mammalian cell membranes and probably exert part of their toxic effect by this mechanism.

Polymyxin B and colistin were shown by Kunin (59) to lose antibacterial activity when incubated with whole cells or homogenates of a wide variety of tissues. This was demonstrated to be due to binding to phospholipids in cell membranes (60).

Further studies by Kunin & Bugg (61) and Craig & Kunin (62) demonstrated that these compounds, when given intramuscularly to rabbits, accumulate in high concentrations in all tissues in two forms. One is a loosely associated tissue form that is extractable in aqueous solution and continues to exhibit antimicrobial activity; the other is a tightly bound form released only by mild acid hydrolysis of chloroform extracts of tissue.

Continuous administration of the drugs does not result in accumulation in serum, but is associated with very high and increasing concentrations in tissue. Toxicity (renal failure and death) is observed once the tissues become saturated with drug. Release of tissue-bound drug is very slow and not complete even at five days after the last dose is given. Polymyxin accumulates both as loosely and tightly associated drug, but colistin accumulates mainly in the tightly bound form. The peculiar effect of colistin may be due to the fact that it is prepared as methane sulfonate derivatives rather than as pure colistin. Methane sulfonation of colistin is used to reduce toxicity by covering several of the free amino groups of the drug. It also appears to decrease antibacterial activity and membrane binding as well. Once injected into the body, the methane sulfonate compound is partly excreted unchanged (accounting for its better renal clearance than polymyxin B) and hydrolyzed into more lightly substituted colistin. Colistin persists mainly in the tightly bound form in tissues, presumably because the methane sulfonate derivative is excreted and does not remain behind.

Membrane binding of the polymyxin antibiotics appears to account for the cumulative toxicity of these drugs, and also may explain why they are much less effective in vivo than in vitro. They appear to work well in the urine and on exposed surfaces, but they have been disappointing in the treatment of systemic gram-negative infection. This may be due to inactivation in vivo by necrotic tissues and pus. Bryant & Hammond (63) have recently shown that 1 ml of purulent sediment will bind and inactivate 1500 μg of polymyxin B or colistin sulfate.

Amphotericin B also binds to cell membrane. Butler & Cotlove (64) demonstrated that binding of amphotericin B by human erythrocytes resulted in potassium leakage. This effect is abolished in the presence of serum. They postulate that sterols present in serum bind the drug and thus exert a protective effect. Erythrocytes from patients treated intravenously with amphotericin B show no direct evidence of increased permeability to potassium during incubation in vitro and have normal fragility. It is well known, however, that one of the major nephrotoxic effects of amphotericin B is renal potassium loss. It seems reasonable to suggest that the renal tubular cell exposed to amphotericin B in newly formed urine is not protected by serum and therefore results in potassium loss. Another explanation of specific nephrotoxicity of amphotericin B has been offered by Weissmann (65). His work suggests that renal lysosomal membranes are relatively rich in cholesterol compared to hepatic lysosomes and are therefore more sensitive to the action of the drug.

Tetracyclines bind to areas of new bone formation, are concentrated by some tumors, and accumulate in areas of inflammation, such as acute myocardial infarcts. They also have been shown by fluorescence studies to bind to mitochondrial membranes (66). In recent studies conducted in our laboratory (67), both tetracycline and minocycline were shown to bind to membranes of human erythrocytes. The effect is diminished in the presence of serum. Minocycline binds more extensively than tetracycline. The mechanism by which they bind is unknown as yet, but tetracyclines have been reported to bind to a variety of metalloproteins, such as catalase, NADH-cytochrome oxireductase, and pancreatic lipase. This is believed to be due to their metal chelating properties.

### *Binding to Nonsoluble Intracellular Substances*

Aminoglycoside antibiotics are inactivated when incubated with homogenates of normal tissues (52). The effect appears to be best explained as nonspecific electrostatic binding to insoluble negatively charged intracellular substances. The effect is not temperature dependent and much of the drug can be released by high concentrations of protamine sulfate, heparin, and electrolyte solutions. Acetone-extracted tissues, free of lipids, bind the aminoglycosides, indicating that the mechanism of their inactivation is quite distinct from that demonstrated with polymyxins. Binding to tissue homogenates is greatest with neomycin, followed by kanamycin, gentamicin, and streptomycin. This correlates well with the number of free amino groups and the relative toxicity of the drugs. For example, neomycin has six, kanamycin has four, and gentamicin has three free amino groups while streptomycin has only two guanido groups.

Binding of aminoglycoside antibiotics by tissues would ordinarily be of little interest since these drugs enter cells poorly and therefore would not be subject to much inactivation. Bryant & Hammond (63), however, have shown that 1 ml of pus will inactivate 700 μg of gentamicin. Presumably necrotic tissue will do the same and interfere with local activity of the drug. The effect is rather nonspecific since gentamicin and other basic antibiotics are absorbed and inactivated by feces (68) and even by the mycelium of fungi that produce these drugs or other antibiotics (69). One wonders how neomycin can be effective in reducing the bowel flora if it is inactivated by feces. Perhaps this is because relatively enormous quantities are given.

*Pseudomonas* is specifically protected from the action of gentamicin and other aminoglycoside antibiotics by $Ca^{2+}$ and $Mg^{2+}$ in the medium (70). The drug is not bound or inactivated by these divalent cations, but the organism is protected. It is possible that $Ca^{2+}$ or $Mg^{2+}$ play permissive roles in the binding of these drugs to tissue sites.

### *Binding to Intracellular Parasites*

Erythrocytes infected with malaria parasites exhibit a remarkable capacity for accumulating chloroquine and other structurally related drugs (71). These drugs rapidly enter uninfected erythrocytes, but the process is markedly increased in parasitized cells. There appears to be a specific receptor(s) on the parasite for the drug. In addition, accumulation is further enhanced in the presence of pyruvate, lactate, glucose, or glycerol, suggesting an active transport process.

### *Binding to Soluble Intracellular Proteins*

In recent years several groups of workers, particularly Litwack, Ketterer & Arias (72), have described a group of soluble proteins in rat liver that bind anionic compounds. Arias and his group, working with bilirubin and sulfobromophthalein, described them as the Y and Z proteins. These workers soon realized that the Y protein was mainly responsible for all of these properties and introduced the term "ligandin" to identify the major binding protein. These are basic proteins with a molecular weight of 44,000, containing two identical subunits of 22,000 daltons each. Recently, Kirsch et al (73) demonstrated an identical protein in rat kidney and found that it bound the same substrates as the hepatic protein, including the antibiotic penicillin G. They also showed that sulfobromophthalein and probenecid competitively bind to the same protein. Hepatic ligandin also binds glutathione and is identical to glutathione transferase B (74).

Kornguth et al (75) examined the ability of rat hepatic ligandin to bind antibiotics and were able to demonstrate specific binding of cephalothin, cephalexin, tetracycline, penicillin G, chloramphenicol, and nitrofurantoin, but not erythromycin or polymyxin B. Rat liver ligandin bound these drugs to about the same extent and in the same order as did rat serum albumin. It was therefore postulated that ligandin may play an important role in the hepatic uptake of these drugs from serum and that these compounds as well as other anionic drugs, such as probenecid, may compete with each other for the binding site. Tetracyclines differ from all other antibiotics in that they are also bound by a second major protein in liver of lower molecular weight than ligandin.

Working with human erythrocytes, Kornguth & Kunin (67) have been able to demonstrate binding and inactivation of penicillins and tetracyclines by hemoglobin. This may account for some sequestration of these drugs in erythrocytes when they are administered to humans, and possibly limits the efficacy of these drugs in infected hemorrhagic lesions. In addition, as with liver, tetracyclines were shown to bind to another protein in erythrocytes. This has been identified as carbonic anhydrase C. Binding of tetracyclines by carbonic anhydrase is augmented in the presence of small amounts of copper or zinc ions. Acetazolamide blocks tetracycline binding to carbonic anhydrase. Surprisingly, however, tetracyclines do not interfere with enzymatic activity.

These studies indicate that rat ligandin in liver and kidney, as well as hemoglobin and carbonic anhydrase, all of which are intracellular proteins, exert a marked effect on anionic antibiotics and may influence their distribution and sequestration in tissues. The liver is a major excretory site of many antibiotics and presumably rapidly turns the drugs over from the binding sites. The erythrocyte, as shown in Figure 1, is a "dead end" cell metabolically, and presumably takes in and releases the drugs from the binding sites in relation to concentrations in the extracellular fluids.

Uptake of anionic compounds by the gastrointestinal tract may also be influenced by a binding protein described by Ockner & Manning (76) in intestinal epithelial, myocardial, adipose, and renal cells. This protein of about molecular weight 12,000 binds fatty acids and appears to be similar to the Z protein described by Arias. It is likely that an analogous protein is present in human intestinal cells. Anionic antibiotics bind to proteins in a manner so similar to bilirubin and fatty acids that it is not unreasonable to postulate that they are also bound by these newly described cellular proteins.

The current status of cellular binding of antibiotics is shown in Table 1. It is expected that this list will grow longer as more information is gained about this phenomenon in future years.

## CONCLUSION

It is now known that antibiotics bind to serum proteins, membranes, soluble intracellular proteins, and insoluble intracellular macromolecules, depending upon their charge and configuration. In some ways these effects appear to mimic the mechanisms whereby they also act against microorganisms. Binding to serum proteins, cells, and tissues not only affects the distribution and uptake of the drugs, but may result in considerable interference with antimicrobial activity. Since antibiotics resemble other drugs and metabolites in the manner in which they are handled by the body, much can be learned by analogy with metabolism and distribution of other drugs. Competition by other drugs and metabolites such as bilirubin and free fatty acids for these binding sites should affect the manner in which they are handled by the body. Antibiotics are unique among pharmacologic agents in that they only incidentally affect homeostatic mechanisms. These incidental effects, however, do influence their more specific role in inhibiting pathogenic microorganisms and therefore must be clearly comprehended if we are to understand how they may best be used in man.

**Table 1** Summary of the major cellular binding sites of antibiotics

| Binding Site | Drug |
|---|---|
| Membranes | |
| acidic phospholipids | polymyxin B and colistin |
| sterols | amphotericin B and nystatin |
| metalloproteins (?) | tetracyclines |
| Insoluble intracellular components | |
| nucleic acids, acidic proteins (?) | aminoglycoside antibiotics: neomycin > kanamycin > gentamicin > streptomycin |
| Soluble intracellular proteins | |
| ligandin and proteins<br>hemoglobin<br>carbonic anhydrase [a] | penicillins, cephalosporins, tetracyclines, nitrofurantoin, chloramphenicol<br>tetracyclines |

[a] These proteins are present in rat liver, kidney, and other tissues; analogous but different proteins bind these drugs in human tissues.

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# THE RELATION OF PSYCHOLOGICAL STRESS TO ONSET OF MEDICAL ILLNESS

❖7200

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## INTRODUCTION

Physicians and related health-care providers have become increasingly aware of the importance of psychological factors in the course and outcome of medical illness. However, there is much less agreement about the influence of psychological factors on the onset and development of medical illness. This chapter focuses on the relationship between psychological stress and the onset of medical illness.

There are a multitude of psychological factors such as personality, psychodynamics, psychopathology, previous family history, psychological stress, physiological stress, and the like that might be considered. This chapter specifically focuses on psychological stress. We must be careful to delineate what we mean by stress inasmuch as this concept is defined differently by leading experts (1–3).

One model of psychological stress postulates an event or situation that precipitates an emotional response such as anxiety or depression (4). The events must be appraised as harmful, threatening, challenging, and relevant to the individual in order to elicit the emotional response (5). This model is also concerned with the potential role of coping and adaptation in determining the intensity and type of emotional response (6). In sum, this model defines psychological stress in terms of emotional responses.

The second model holds that certain stimulus situations impose a psychological stress on an individual because they elicit attempts at adjustment (7). In other words, this model defines psychological stress in terms of the occurrence of certain stimulus situations rather than in terms of emotional response. If individuals are similar in how they rank various life events in terms of adjustment, then we may postulate that such events impose equivalent psychological stress for different individuals.

Recent work has shown that individuals of the United States, Norway, Finland, Japan, Denmark, and Sweden tend to rate a list of 40–50 stimulus life events in almost exactly the same way (8–10). For example, the death of a spouse is uniformly seen as an event that would cause a tremendous amount of adjustment, whereas a vacation would not. This second model therefore would imply that the amount of adjustment required defines the stressfulness of the event.

The relationship of psychological stress to mental illness has been extensively studied and many current reviews are available (7, 11). The relationship of psychological stress to certain physical illnesses is less well known and documented. Two physical disease clusters—heart disease and cancer—are leading causes of death in the United States. Psychological stress has been implicated in their onset over the years. Recent literature has been particularly intriguing.

This chapter focuses specifically on the relationship of psychological stress to the onset of cardiovascular and neoplastic diseases. Each of the disease categories is then considered from the two approaches to definition of psychological stress: emotional responses and life event stimulus situations. Finally, conclusions and suggestions for research and practice are offered, based on the preceding review.

## HEART DISEASE

This section concentrates on those psychosocial variables that qualify under the rubric of "stress" as defined above. These include disturbing life changes on the stimulus side of the equation and unpleasant effects on the response side. We have specifically excluded consideration of predisposing personal traits such as the "coronary-prone behavior pattern," which has been well established as a risk factor to coronary heart disease.

### *Anxiety and Depression Studies*

Classical symptoms of anxiety include tenseness, sleep problems, irritability, numbness, restlessness, pains, and vague fears (12). Symptoms of depression include sadness, sleep problems, loss of appetite, irritability, restlessness, fatigue, and lack of interest (12). Since the symptoms overlap a great deal, we will view anxiety and depression together as emotional responses indicative of psychological stress.

Thiel et al (13) studied 50 patients with myocardial infarctions (MI) and 50 age-matched, healthy, nonpatient controls. The patients with MI scored significantly higher on anxiety and depression scales than the controls. Specifically, they reported significantly more feelings of nervousness, sleep disturbances, and shortness of breath. Thiel and his co-workers probed further in interviews with the patients and controls. They found that many of the MI patients reported being particularly anxious and depressed prior to their MI. Unfortunately, the significance of these findings may be overestimated, since the controls were healthy subjects rather than patients with other diseases. In addition the experience of MI may have biased the recall of anxiety and depression symptoms.

Two prospective studies have confirmed the importance of sleep disturbances prior to MI. Friedman et al (14) studied thousands of Health Plan records in the

Kaiser-Permanente medical program. The patients had responded to a 155-item questionnaire earlier in the program. Lack of energy, feeling blue, and many minor physical complaints were reported significantly more often by those who later developed MI than by persons who remained free of heart disease. Friedman also found that persons who reported trouble falling asleep or staying asleep were more likely to be in the group that later developed MI than in a control group remaining free of coronary heart disease (CHD). However, when all the subjects who had symptoms at initial exam suggestive of possible myocardial ischemia were removed from the analysis, the differences between those who did and did not later develop MI were diminished.

Thomas & Greenstreet (15) administered several biographical and psychological questionnaires to first- and second-year medical students. They contacted these individuals, or their families in cases of death, 11 to 27 years after graduation to determine their health status. The investigators found that medical students who had reported being fatigued upon awakening incurred MI proportionately more often in later years than those students who did not have this sleep complaint.

Kavanagh & Shepard (16) also noted that increased tiredness and a deterioration in general health was reported in the week prior to MI by 102 survivors of the event. This study, however, had no control group.

Greene et al (17) reported that sudden coronary death was frequently preceded by a period of depression followed by an abrupt change to a state of arousal. The arousal was marked by increased work, anxiety, or anger. The MI followed shortly upon the reactive arousal period. These clinical results were limited by the lack of specific measures of depression, anxiety, and anger, as well as by the fact that no control group was included.

A prospective Israeli study (18) reported that a three-item anxiety index was prospectively associated with the incidence of angina pectoris (AP). High-anxiety subjects developed twice the incidence of AP as did low-anxiety subjects. This was a statistically significant difference. However, in this study the anxiety assessment was not predictive of MI.

A controlled study by Eastwood & Trevelyan (19) screened 2200 English citizens who were on medical practitioners' lists. Psychological screening inventories and interviews were administered to identify persons with psychiatric problems. Chronic mild anxiety and depression were the main symptoms of the group so identified. They were matched with control subjects from the same medical lists who did not manifest these symptoms. Both groups were given thorough cardiological examinations. Significantly more diagnoses of "possible and probable CHD" were found in the anxiety-depression group than in the control group. This difference held true for both males and females.

In sum, recent clinical and prospective studies suggest that either newly-evidenced anxiety and depression or exacerbated chronic anxiety and depression may heighten the probability of onset of CHD and particularly of AP. However, the evidence fails to distinguish whether these manifestations of psychological stress are long-term precursors or short-term precipitants of CHD. Also, it has not been established that either of these manifestations interact with recognized risk factors such as high serum cholesterol, high blood pressure, cigarette smoking, obesity, or the coronary-prone behavior pattern (Type A).

### *Life Event Studies*

In the following studies, and those reported later in the section on cancer, the Schedule of Recent Experiences (20) or a similar scale has been used. Subjects indicate the occurrence of life events (such as loss of job, buying a house, death in the family, and so forth) for given periods of time such as the past 6 months, 7–12 months ago, 13–18 months ago, and 19–24 months ago. Each event is assigned Life Change Units (LCUs) according to previous studies and the LCUs are summed for each time period. A typical listing of events and LCU is given in Holmes & Masuda (21). The sum of occurrences and/or the sum of LCU can be considered an estimate of psychological stress.

Rahe & Lind (22) obtained a list of 67 persons who had died a sudden cardiac death in Stockholm, Sweden, during a three-month period in 1968. A total of 39 next-of-kin participated in this study. They completed the Schedule of Recent Experiences (SRE) on behalf of the deceased. The investigators found a statistically significant increase in the total LCU for the sudden-death patients in the year prior to death, compared to the two years before that year. The final six months before death showed the highest levels of LCU. These findings held true for patients who did and did not have prior CHD histories. This seemingly impressive finding is greatly marred by the lack of a control group, since the increase in LCU as time approached the present could merely be a result of better memory in the next-of-kin for recent as compared to past periods.

Three very similar studies have presented comparable findings. Theorell & Rahe (23) studied 54 post-MI patients. Again there was a significant rise in the total LCU in the six months preceding the MI. This was true only for those patients with no previous history of MI. Those with a prior history of MI had higher life change scores both in the prior six months as well as before that time. In contrast, another uncontrolled study of 30 male post-MI patients (24) found no significant difference in the mean level of life change in the final six months. Rahe et al (25) studied 279 survivors of MI. They, too, completed the SRE. Subjects were divided into two groups: one MI group was characterized by a good health history for the two years prior to MI, the other by a poor health history for the same period of time. The group with illness prior to the MI had twice the total amount of life change over each six-month period in the two years as did the group without an illness history. Some of the difference between these groups may have resulted from the fact that several SRE events are health changes. Hence the group with prior illness would be expected to have a higher life change total than the healthier group. Both groups, however, did show a significant rise in LCU during the final six months prior to the MI.

The above studies are suggestive but not conclusive. They are severely handicapped by their retrospective designs. The results are highly dependent on recall, which may be influenced by the occurrence of MI. In addition, the studies all lack comparison groups that would control for type and occurrence of illness as well as for decreases in recall as a function of elapsed time.

Theorell, Lind & Floderus (26) attempted to overcome these limitations using a prospective design with 6579 male construction workers in Sweden. Follow-up reviews of medical records were completed for all workers 12–15 months after the completion of the SRE. Subjects with first MI events ($N$=32) were compared to all other subjects after excluding those with cardiovascular disease known at intake. There was no significant difference between the mean levels of life change between the MI group and all others.

Although these life change studies might suggest some causal role of stressful life situations in the onset of heart disease, other explanations for the reported results can be offered. Selective or diminished recall of life events over extended periods of time would account for the significant difference in life change totals of the final six months prior to MI compared to results from a year earlier.

A person-specific or environmentally specific adjustment for a given life event may be more important than the generalized adjustment value assigned from studies of large populations of persons, most of whom have not recently experienced the events they are rating. This possible alternative is given credence by Lundberg, Theorell & Lind (27). They found that the sum of an individual's own retrospective assessments of distress associated with each life event was, in fact, more strongly discriminating between the MI and the comparison group than the use of individual ratings of adjustment demanded by the life events or average assessments by the total group of either distress or adjustment. Thus, the occurrence of events commonly believed to demand adjustment may be less important in the onset of heart disease than the occurrence of events that the individual finds personally upsetting.

In other words, systemic emotional arousal (2) engendered by events relevant to the individual (5), or in which the individual is engaged/involved (28), may constitute the most important aspect of psychological stress in the onset of heart disease. This suggestion finds some support in a recent study of patients with congestive heart failure.

Perlman et al (29) noted four studies examining the relationship between emotional factors and the onset of congestive heart failure. Two studies seemed to indicate a strong emotional precipitating factor and two did not. One of the reasons suggested for this discrepancy in results was "different definitions of emotional stress." Another was the "depth of patient and family interviews." Perlman et al interviewed each of 105 congestive heart failure patients for 1½–2 hr. An age- and sex-matched group of 50 consecutive patients with no history of congestive heart failure was also interviewed. The interviewer was unaware of the patient diagnoses. Relatives were interviewed and asked the same questions as the patients.

The definition of emotional/psychological stress for this study was unique. To quote the authors (29, p. 2),

> For this study an emotional or psychological factor was defined as any event or circumstances that represented trauma or produced a strong emotional reaction in the patient. Only events and reactions that occurred not more than 3 days before the onset of symptoms and hospitalization were considered. Chronic anxiety states of mind . . . were not included. Changes in the patients' affect during the relating of the event and the effects it had on his behavior before hospitalization were more crucial than the nature of the event itself.

This definition encompassed each aspect of Mason's (1, 2), Lazarus's (5), and Singer's (28) components of psychological stress. Although the Perlman study was retrospective, the definition and results are instructive. Fifty-one of the 105 congestive heart patients (49%), but only 12 of 50 (24%) control patients, experienced a clearly identifiable emotional event in the three days preceding hospitalization. This difference was significant at the 0.01 level. In this case the period of recall was so short that memory should not have been a confounding factor in the results. However, there may have been a differential propensity to report such events between the two groups rather than an actual difference in occurrences.

No matter how one examines the data, it seems that both perspectives of psychological stress suggest an important role for psychological distress in the onset of heart disease. Until more evidence of a substantial and controlled nature is available, we cannot say how important psychological stress is, compared to physiological risk factors or to other psychosocial factors such as the coronary-prone behavior pattern. It may well turn out to be the case that psychological stress is a catalyst for other risk factors rather than a true participant in the disease process. On the other hand, chronic and repeated exposure to stress may have direct physiological consequences that play a role in the etiology of heart disease (30).

### *Cancer: Anxiety and Depression Studies*

Early in this chapter we noted that one perspective on psychological stress suggested that anxiety and depression would indicate the presence or operation of psychological stress on a person. Review of the recent heart disease literature suggested that persons suffering from anxiety and depression were more likely to experience the onset of CHD than persons who were not anxious and depressed. This kind of more carefully controlled comparison study has not been done for cancer. The lower incidence of cancer and the tremendous difficulty inherent in trying to ascertain the patient's psychological state prior to the development of cancer has discouraged prospective studies along these lines. However, it is worthwhile to examine some of the conclusions made by researchers.

LeShan (31) reviewed literature published from 1902–1957. The great majority of studies were clinical in nature and based on the personal experiences of the reporting physicians. One of the main themes of all these studies was that depression, hopelessness, and undischarged grief seemed to be significant precursors of the discovery of cancer. LeShan also pointed out that many studies suggested that depression and hopelessness were reactions to the unreplaced loss of an emotionally close relationship. Studies conducted by LeShan & Worthington (32–34) seemed to indicate that this loss of a particularly intense personal relationship could be placed prior to the probable onset of the disease. The development of the depressive symptoms seemed to occur just prior to the appearance of the cancer. However, all of the studies reviewed by LeShan were retrospective and the vast majority were uncontrolled. Hence these conclusions are more provocative than conclusive.

Greene (35) reported on a series of three studies with leukemia and lymphoma patients that he and his co-workers had conducted over several years. Over one hundred patients had been studied by the time of Greene's summary report. He and his workers felt that depressive and anxious reactions to losses and separations were symptoms particularly associated with the onset of leukemia or lymphoma.

In their initial comments to a paper devoted to ego defenses in the etiology of cancer, Bahnson & Bahnson (36) noted that loss and depression seemed to be significant antecedents to the onset of cancer. However, they further cautioned that these conditions and emotional states may be necessary but not sufficient conditions for the onset of malignant neoplasms.

Hagnell (37) reported on a prospective study of the personality of 2550 Swedish citizens who were followed for ten years. He found a significant difference in the premorbid personalities of those who did and those who did not develop cancer. Unfortunately, the assessment procedure was based on an unusual personality theory that is not comparable to any other system. However, the "substable" personality, characteristic of those who developed cancer, had a major component related to depression. The "substable" persons tended to lose energy and withdraw emotional expression when depressed. A liberal interpretation suggests that this type of person suffered from a more severe form of clinical depression than those persons characterized as "superstable" (cool, analytical, and aloof).

At least one, if not the only, prospective study of the role of anxiety and depression in the onset of cancer has been reported recently. Thomas & Greenstreet (15) followed up on 1076 graduates of the Johns Hopkins Medical School. Initial testing was completed in the first two years of medical school. The follow-up data was collected 11–27 years after initial testing. They found that those nine who later developed cancer had consistently lower depression, anxiety, and anger at initial testing than those students who died of suicide, or developed mental illness, hypertension, or coronary occlusions. These students also were significantly lower in anxiety and depression than the normal controls (disease-free at follow-up).

This prospective finding clearly echoed the findings of Kissen (38); Kissen, Brown & Kissen (39); and Abse et al (40). These investigators had consistently found that cancer patients (and lung cancer patients in particular) had significantly lower neuroticism scores than other patient groups as well as normal controls. These studies tested patients admitted for diagnostic work-ups. Testing was done prior to diagnosis. Control patients were those whose work-ups resulted in nonmalignant diagnoses. The neuroticism measure used by these investigators is equivalent to an assessment of physical complaints and anxiety.

Thus, the results of the prospective and carefully controlled studies contradict the clinical and retrospective uncontrolled studies. Both theoretical (36) and descriptive (38) explanations have been offered that might resolve the contradiction. However, the explanations have not been replicated in other settings, let alone put to the test of a prospective study.

From the point of view of psychological stress in the onset of cancer, it seems to us that the literature points very strongly to the need for prospective studies that include at least three elements for prediction, namely, assessments of psychological factors, physiological risk factors, and longitudinal stimulus event histories. Only then may it be possible to unravel the role of psychological stress in the onset of cancer.

### *Life Event Studies*

In his review of 75 studies, LeShan (31) concluded: "The most consistently reported, relevant psychological factor has been the loss of a major emotional relationship prior to the first-noted symptoms of the neoplasm." A closer examination of the studies he reviewed in coming to this conclusion indicated that these losses might have occurred anywhere between six months and eight years before the probable onset of symptoms. Kavetsky, Turkevich & Balitsky (41) reported similar findings and conclusions by many Russian clinicians. They also reported that the Russians tended to explain these findings in terms of endocrine or metabolic changes that occurred under conditions of psychological stress.

In the section on anxiety and depression, we noted two studies by LeShan & Worthington (32, 34) that indicated that a significantly greater proportion of cancer patients than controls (both disease-free and noncancer patients) revealed a history of a loss of a particularly intense emotional relationship prior to the diagnosis of cancer. This deduction was made on the basis of a projective test that was administered to all the research subjects.

These suggestive results led to an epidemiological study of mortality rates for groups of people that could be expected to differ in terms of the loss of an important emotional relationship (42). Widowed persons were predicted to have the highest cancer mortality rate; divorced persons the next highest; then married persons; and finally single persons. LeShan then reviewed several reports of female cancer mortality rates and regrouped the data to reflect age-adjusted mortality rates for the widowed, divorced, married, and single groups. In the five studies he reviewed, LeShan "found no statistical studies whose results were inconsistent with the hypotheses." These results seem quite suggestive. However, it is important to note that the results were only for women and were based on data from 1900–1932. We do not know if this finding would continue to hold true in more recent times or if it applies to men. This might be a fruitful area of investigation.

Greene's (35) summary report of three studies involving 100 leukemia and lymphoma patients suggested an etiological role for anxiety and depression. The most common precipitants for these reactions were death and threat of death or separation from parents, spouse, children, or other significant persons. The retrospective interview examination covered the four-year prodromal period before the apparent onset of the cancer. The loss experience most often occurred around 12 months prior to onset.

Bahnson & Bahnson (36) also reported on a series of studies they had conducted. Ninety-nine cancer patients suffering from various cancers were studied by means of an unstructured interview. Thirty-eight normal males served as control subjects. The interviewers were aware of patient diagnoses. The implications of the results of this study seemed to be that the cancer patients experienced an unresolved loss of a dependency relationship one to two years prior to the onset of the neoplasm.

Thus, LeShan (42), Greene (35), and Bahnson & Bahnson (36) all reported similar findings. Their data suggested that the loss of a particularly intense dependency relationship occurred shortly before the onset of the cancer.

A more direct life event approach was applied by Schmale & Iker (43). They reported on a series of three investigations with a total of 51 women. The women had been referred for a cone biopsy due to positive Pap tests. They had no gross evidence of cervical disease. An open-ended interview and psychological tests were administered before the biopsy results were known to the subject, her physician, the pathologist, or the investigators. The biopsy results indicated that 19 women had cervical cancer.

The interview protocols were rated for the presence of feelings of hopelessness in response to a life event in the six months prior to the first positive Pap smear. According to this criteria, 18 subjects were predicted to have cancer. Thirty-three were predicted to not have cancer because they had not developed the hopelessness reaction. Eleven of the 18 predicted to have cancer did, in fact, have it. Of the 33 predicted not to have it, 25 did not. An error in interpretation led the researchers to conclude that these results were nonsignificant. However, our own calculations show that the predictions were significantly accurate at a high level of confidence (chi-square-6.76, $df=1$, $p$ less than 0.01). In addition, those who actually had cancer had significantly higher depression scores on a personality test.

On the other hand, Muslin, Gyarfas & Pieper (44) studied 165 women admitted for breast biopsies. A questionnaire was completed and an interview was conducted before the results of the biopsy were known to subjects or investigators. The interview was rated for the presence or absence of "a permanent loss of a first degree relative or other person whom the subject specifically stated was emotionally important to her." Subjects were grouped into pairs of benign and malignant lesions with each pair being matched for age, race, marital status, and socioeconomic status. It was possible to produce 37 matched pairs by this method.

The interviews revealed an excess of 17% more separation losses among the cancer subjects while the questionnaire revealed only an 8% excess in comparison to the matched controls. The differences between loss rates of the cancer patients and controls was nonsignificant.

Grissom, Weiner & Weiner (45) studied 30 lung cancer patients, 30 emphysema patients, and 30 disease-free veterans. Group smoking histories were matched by subject selection procedures. The SRE and the Tennessee Self Concept questionnaires were administered to all of the subjects. The LCU totals for the year prior to admission to the Veterans Administration hospital for diagnostic purposes were not statistically different among any of the groups.

Thus, for lung and breast cancers there has been a lack of definitive evidence that increased life event stress plays a role in the onset of the neoplasm, while there has been some consistent suggestion that life event stress may be related to the onset of cervical cancer, leukemia, and lymphoma. Since all of the studies were retrospective, Grinker's (46) caution seems pertinent: "There is always danger of attributing characteristics to a particular event if a disease process such as carcinoma develops somewhere in the adjacent time span. . . ." On the other hand, we do not fully concur with Crisp's (47) or Brown's (48) suggestions that clinicians and researchers should be more concerned with course and outcome than with etiology. Their position has fewer inherent difficulties from a research and rehabilitation standpoint, but it tends to rule out control and prevention.

## IMPLICATIONS AND CONCLUSIONS

Research and clinical evidence suggests that psychological stress may be important in the onset of heart and neoplastic diseases. Yet most of the evidence is based on retrospective and/or uncontrolled studies. The few prospective studies that have been undertaken seem to contradict or diminish the significance of the retrospective findings, particularly for cancer studies. Overall, the results of studies on the relationship of psychological stress to the onset of medical illness have suggested a complex interaction of factors rather than a simple causal link.

This complicated state of affairs does not seem to hold with respect to the course and outcome of cancer or cardiac patients. Depression, hopelessness, and anxiety have been shown to adversely affect the course and outcome of cancers in several recent studies (48–53). Similar findings have been reported for cardiac patients (54) and for patients with congestive heart failure (29). These course and outcome studies are implemented more easily and at less expense than studies on etiology and onset. They suggest a strong role for psychiatric consultation and liaison in the rehabilitation of cardiovascular and neoplastic patients. But we are left with the question of what to do to prevent the disease in the first place.

At this stage of research in the area, the most appropriate conclusion is that more research is needed. Long-term prospective studies with carefully defined and selected controls are needed. Consistent and replicable assessments and definitions of psychological stress would help alleviate difficulties in interpretation of results. Simultaneous testing of psychological, physical, and historical factors in a repeated prospective design would augment the explication of the interaction between stress and disease onset. Caution, reliability, and scientific rigor have been neglected too long in this important area.

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# MEDICAL MANAGEMENT OF VENOUS THROMBOSIS[1]

◆7201

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## INTRODUCTION

Deep venous thrombosis of the lower extremities is a common age-related phenomenon manifest by focal intravascular coagulation in which the mechanism is obscure, the clinical recognition elusive, the recurrence rate high, and mortality unpredictable. The urgency of the physician's concern with deep venous thrombosis clearly lies in the intimate but variable association between thrombi in peripheral veins and death from embolization of these thrombi to the pulmonary arteries—thus the rubric, venous thromboembolism.

Venous thromboembolism is a ubiquitous and frequently lethal complication (50,000 deaths annually) of a broad spectrum of medical and surgical diseases and of sedentary living. Its hazard to a productive life has been masked by a lack of awareness of its true incidence and by the fact that it is not itself a disease, but rather a complication of a host of major disease states. Recent data have documented its remarkable scope, its increasing prevalence in our society, its mechanism of action at the molecular level, and most importantly, have provided new methods of primary prophylaxis that are now available for general use by physicians and surgeons. In short, venous thromboembolism has become a largely preventable complication of disease (1).

Anticoagulants, particularly heparin and the coumarin compounds, are the mainstay of the therapeutic armamentarium in the prophylaxis of venous thromboembolism. Current interest has focused on the newer applications of heparin, particularly at the onset of the risk of intravascular coagulation, while coumarins maintain their value mainly in long-term prophylaxis. Thrombolytic therapy may have an accessory role in the management of massive pulmonary emboli. The practical usefulness of venoms remains to be established, and evidence is not yet available that platelet antiaggregating agents are therapeutic in venous disease. Finally, a variety of physical measures that improve venous flow in the extremities are useful adjuncts, especially when anticoagulant therapy is contraindicated (1).

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## HEPARIN

### *The Heparin Dilemma*

Despite the demonstration of the antithrombotic action of heparin in 1936, the proper use of this compound 40 years later is impeded by a welter of claims and counterclaims (2). There are conflicting statements concerning the unit dose, route, frequency, and duration of therapy. At least three approaches to dosage regulation are practiced: (*a*) a preselected schedule that requires no laboratory control, (*b*) the whole-blood clotting time, and (*c*) a variety of partial thromboplastin times. Recently, several assays based on the reaction involving heparin, activated Factor X (Xa), and antithrombin III have been proposed for the measurement of plasma heparin. Still, there is no agreement, within or between assays, as to the desired therapeutic regulation of heparin therapy.

It is also unclear whether dosage, in adults at least, should be related to body weight, sex, or the severity of the thrombotic process. In addition, there are data to indicate that normal individuals vary widely in their responses to a given dose of heparin. These problems are further compounded by the knowledge that heparin is a molecule exhibiting chemical heterogeneity caused by inherent molecular instability and by polydispersion (3).

Despite the facts that greater purification of heparin has been achieved commercially, the molecular biology of heparin action has become clarified, further knowledge of the pathogenesis of thrombosis has been uncovered, and the criteria for controlled clinical trials have been sharpened, heparin is presently used with less confidence in its efficacy and safety than at the start of World War II.

### *The Clinical Use of Heparin in the Prophylaxis of Venous Thromboembolism*

The physician, when he employs heparin to prevent venous thromboembolism, wishes to choose a dose that is antithrombotic for the selected clinical setting and essentially devoid of the risk of hemorrhage. It is generally accepted on both biochemical and clinical grounds that the amount of heparin required to prevent thrombosis before intravascular coagulation has occurred is less than is needed after thrombosis has developed. It can also be stated that when thrombosis is extensive, more heparin may be required than when intravascular coagulation is minimal. On this basis, one can divide heparin treatment into these categories: low-dose, medium-dose, and large-dose. Of these three regimens, low-dose, the last to come into vogue, has been the most rigorously monitored for efficacy by clinical trial and has the least risk of hemorrhage.

It is now clear that neither specific nor general clotting assays will totally protect the individual patient from hemorrhage. Aside from choosing the correct heparin schedule for the clinical episode to be treated, hemorrhage can best be minimized by careful attention to recognizing coexistent systemic or local abnormalities that may lead to hemorrhage, other concomitant drug therapy that may itself predispose to hemorrhage, such as aspirin or adrenal corticosteroids, and the duration of heparin therapy. Particularly in the medium- and large-dose categories there is a correlation between the duration of heparin therapy and the development of bleeding.

### *Low-Dose Heparin*

RATIONALE Details of the early history of low-dose heparin and the molecular basis for its efficacy have been recently summarized (2). In brief, the anticoagulant action of heparin depends on the presence in plasma of an $\alpha 2$ globulin known as antithrombin III (heparin cofactor, inhibitor to Xa), which inhibits several coagulation proteases, particularly thrombin and Xa. Heparin accelerates the reactions between antithrombin III and these proteases. During coagulation, Factor X operates at the beginning of the final sluice through which essentially all known coagulation activators must pass, converting the zymogen to the activated species Xa, which leads via thrombin to both platelet aggregation and fibrin formation. Biochemical data strongly suggest that the primary physiological target of antithrombin III, in terms of preventing thrombus formation, is Xa rather than thrombin (2). These observations are also consistent with the view that the enzymatic coagulation sequence functions as a biological amplifier; that is, it takes less energy to stop intravascular coagulation at the Xa step than at the subsequent thrombin stage.

Accordingly, it seems reasonable to suggest that if a tendency to thrombosis were treated before intravascular coagulation was initiated, less heparin might be required than if therapy were begun after thrombin formation had occurred. In the former situation, small amounts of heparin will markedly increase antithrombin III reactivity. One excellent clinical situation in which to test this thesis is the postoperative state,since many thrombi begin at the time of operation. It would be, in essence, the presence of small amounts of exogenous plasma heparin, augmenting manifold the activity of antithrombin III to prevent the development of hypercoagulability, that might diminish venous thrombosis in patients undergoing surgery.

CLINICAL TRIALS It was with this rationale in mind that our laboratory collaborated with Mr. Kakkar's group at the Kings College Hospital Medical School, London, in the first exploration of low-dose heparin in surgical patients using $^{125}I$ fibrinogen scanning as a marker of deep venous thrombosis (4). Since then a number of trials of the efficacy of low-dose heparin have been published (5–18). Most of these studies have indicated the effectiveness of low-dose heparin in preventing postoperative deep venous thrombosis, although there has been at least one in which no antithrombotic effect was observed (19), and several in which specific groups of patients did not benefit (5, 7, 14, 18, 20, 21). It is not clear at the present time what the recommended dose should be for general surgical patients: 5000 units two or three times a day subcutaneously. While the higher schedule may be slightly more antithrombotic, at least in deep venous thrombosis (6, 10, 18), there are data to suggest that there is also more bleeding (10). Salzman has emphasized that although hemorrhage in these trials has not been severe, the potential hazard of uncontrollable bleeding exists (22).

To determine whether low-dose heparin in surgical patients prevents lethal pulmonary emboli, Kakkar headed a multicenter and multinational group that recently concluded the largest controlled clinical trial ever undertaken to evaluate an anticoagulant agent (23). Although over 4000 patients were involved, the size of the trial population was not large enough to demonstrate a mortality difference between treated and control groups. Autopsy findings, however, revealed that large pulmonary emboli (those that could have caused or contributed to death) occurred in 22 of the control and in 5 of the treated patients. If one scored all pulmonary emboli, large and small, there were 42 patients in the control and 14 in the treated group. Both these differences are highly significant statistically. Increased bleeding, though not a serious problem, did occur (23).

From these data it can be inferred, though not established, that therapy provided a meaningful survival benefit from a standard dose without need for monitoring and with an apparently acceptable risk of hemorrhage.

These cumulative studies of both venous thrombosis and pulmonary embolism suggest that low-dose heparin has a role in the primary prevention of postoperative pulmonary emboli among patients subjected to general abdominal surgery. Whether the dose should be 10,000 or 15,000 units of heparin daily has yet to be resolved. Moreover, the value of therapy is under question in certain procedures at high risk of thrombosis such as open prostatectomy and major orthopedic surgery. Low-dose heparin is also considered inadequate for patients undergoing surgery during an active thrombotic process.

### *Medium-Dose Heparin*

As correctly emphasized by McNicol, once the low-dose heparin schedule is exceeded there are no acceptable clinical trials of heparin prophylaxis in venous thromboembolism with regard to either efficacy or safety (24). Once venous thromboembolism has occurred, low-dose heparin regimens are inadequate. For patients in whom the extensiveness of the thrombosis as estimated clinically or with laboratory guides is not great, a range of 20,000–60,000 units per day encompasses most of the reports (25–30). In the majority of these studies heparin was given by intermittent intravenous injection. Most of the regimens were in the range of 30,000 –45,000 units per day. Some investigators have used body weight in determining the daily dose, but most have not. Some reports recommend lower schedules for older women, others do not. Although many investigators use some clotting assay as a guide to dosage, there are several who do not. Accordingly, it is difficult to develop specific recommendations based on firm experience. Our interpretation leads us to conclude that if one gives this group of patients 5000 units intravenously every four hours (30,000 units per day), screens adequately for bleeding risks prior to treatment, protects the patient from bleeding risks during therapy by such methods as interdicting all intramuscular injections, and determines the hematocrit once or twice daily, then it is feasible to administer this amount of heparin without monitoring. If an overall assay such as the partial thromboplastin time is used, all it can demonstrate in the majority of, but not all, patients is that heparin is circulating and that the dose is not extreme.

The risk of bleeding will be less during the first 48 hr of this regimen than later on in the patient's course. Accordingly, if after 48 hr there is clinical or laboratory evidence of the cessation of progression of the disease, it would be reasonable to reduce the daily dose to 25,000 units and start the patient on coumarin therapy. It is desirable to allow a six-day overlap of the two anticoagulants after the prothrombin time has reached the therapeutic range (31). At this time heparin may be discontinued and the patient maintained on oral anticoagulants on an ambulatory basis for a period of two to six months. Should recurrence occur during coumarin therapy, it would be necessary to begin again with heparin for two weeks and then return to coumarins. This regimen will minimize serious hemorrhage, but provides no guarantee of an antithrombotic effect. In view of the recent work of Salzman et al, a constant intravenous drip of 30,000 units per day may be safer, although apparently no more efficacious than intermittent intravenous therapy (25). Practical issues may decide whether it would be best to keep the patient on a constant drip, use intermittent intravenous therapy, or provide the 30,000 units a day in divided doses subcutaneously. If the above outlined regimen fails to prevent further thromboembolism, either the amount of heparin was insufficient or some already present thrombotic material broke off from the vein and reached the pulmonary arteries. A distinction between these two interpretations is not always easy, but it is important to recognize that occasionally pulmonary embolism following initiation of heparin therapy is not due to a failure of the drug, which never had the capability of preventing fracture of a venous thrombus with subsequent embolization to the lung. Confronted with such a situation, however, one could maintain the regimen, increase the dose from 30,000 to 45,000 units a day, or surgically obstruct the inferior vena cava by one of several available methods.

### *Large-Dose Heparin*

In patients with massive pulmonary embolism as much as 120,000 units per day (intermittent intravenous) has been recommended for the first 24 hr of treatment (32, 33). Others have stated that 60,000 units are adequate (34, 35). Here again there are no guides from clinical trials and such large doses should be reserved for florid cases in which pulmonary emboli are causing cardiovascular instability or shock. These high doses should be reduced to the medium-dose range within 24–28 hr after the institution of therapy. The risk of bleeding on the highest regimen is reported to be low (32, 33), and there appears to be little purpose in doing clotting assays (36). These observations may merely show that large amounts of heparin may not produce hemorrhage within a few hours, rather than indicating the amount of heparin actually required to prevent further thrombosis in these patients.

Several reasons have been advanced for the need for large doses. One is that under certain conditions a tripling of the control clotting time has been necessary to prevent stasis thrombi (37). It has been suggested that large amounts of heparin are required to "turn off" accumulated thrombin, following which it is possible to obtain a therapeutic effect with less heparin (37). It is also claimed that in animals, large amounts of heparin are necessary to prevent platelet accretion on a fresh embolus, which in turn will prevent propagation of thrombotic material (38). Finally, it has been suggested that these large doses are helpful in reversing the bronchoconstriction that is often a concomitant part of pulmonary embolism both in animals and in man (38).

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# BEHAVIORAL AND BIOCHEMICAL INTERRELATIONS IN ALCOHOLISM

❖7202

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## INTRODUCTION

Exploration of the interrelationships between biochemistry and behavior is a relatively new venture in biomedical science. We have just begun to understand some of the complex neurochemical events associated with very basic behavioral phenomena such as sleep, memory function, and affect. However, identification of a specific chemical derangement as the basis of alcoholism or any other serious behavior disorder (such as schizophrenia) still appears unlikely. Indeed, hypotheses that ascribe disease to any single determinant are being replaced by multideterminant causation models. There are a number of behavioral concomitants of alcohol abuse and alcoholism that appear to have significant biochemical interrelationships that can be studied with current biochemical and behavioral techniques. These include biobehavioral mechanisms of tolerance and physical dependence, the effects of alcohol on sexual function and behavior, the effects of alcohol on hepatic function and its relationship to behavior, and assessment of the contribution of genetic factors to alcohol-related disorders.

Knowledge gained in each of these areas has many implications for the medical management and treatment of the alcoholic individual. Biochemical mechanisms subserving the processes of tolerance and physical dependence must be understood before rational pharmacotherapies can be developed for the treatment of alcohol addiction. Factors underlying tolerance regulate crucial behavioral phenomena, namely the need for an alcohol addict to progressively increase ethanol dosage in order to produce a change in feeling state or behavior that was originally achieved with lower dosage. Physical dependence is often considered one of the most important variables that influence perpetuation of drinking by the alcohol addict. Although physical dependence alone probably cannot explain chronic drinking behavior, it is nonetheless an important process that partially defines the addictive state. Alcohol abuse frequently results in impotence in males, and gross derangements in sexual behavior such as forcible rape often are also linked to excessive drinking. Since alcohol-induced derangements in sexual function and behavior are so common, a better understanding of biochemical-behavioral relationships of the effects of alcohol on sexual processes is necessary. Alcohol-induced hepatic disease, principally Laënnec's cirrhosis, is one of the leading causes of death in major metropolitan areas of the United States. Morbidity and behavior dysfunction associated with alcohol-related hepatic disease take an enormous toll in human resources and well-being. Models have recently been developed that may advance our understanding of the determinants of liver disease associated with alcohol abuse. Although the nature-nurture question of the genesis of alcohol-related disorders and most other behavior disorders may never be fully answered, the relative strength of genetic determinants and their possible manipulation through advances in biochemical pharmacology can be examined. Space limitations require a brief and cursory discussion of these topic areas. For more detailed information, the reader is urged to examine the specific papers listed in the reference section at the end of this chapter.

## TOLERANCE

Two major factors are believed to account for development of tolerance for ethanol. The first involves metabolic adaptation and the second is associated with adaptive changes in central nervous system (CNS) function. Studies of metabolic adaptation have been easier to carry out than examinations of changes in CNS function. Consequently, more data are available concerning metabolic adaptation than CNS adaptive changes related to tolerance.

Assessments of metabolic adaptation have been carried out largely by measuring blood alcohol disappearance rates in normal human subjects and alcohol addicts. However, this approach has been criticized because blood ethanol disappearance rates may not parallel rates of ethanol metabolism (1). Since alcohol is distributed in body tissue on the basis of water concentration, rates of blood ethanol disappearance may vary as a function of total body weight as well as relative composition in body weight (ratios of lipid to protein). For example, alcohol disappearance rates would be expected to be more rapid in obese individuals. Therefore, it has often been necessary to employ subjects as their own controls in studies of the effects of alcohol intake on induced changes in alcohol metabolism.

Studies in which alcohol addicts were examined prior to and following a period of experimentally induced intoxication revealed that a significant increase in rate of ethanol metabolism occurred following heavy alcohol intake (2). Alcohol addicts almost doubled their rate of ethanol metabolism following heavy drinking. Measurements included both the disappearance of ethanol in the blood after peak blood alcohol levels were achieved and the appearance of $^{14}CO_2$ following administration of isotopically labeled ($^{14}C$) ethanol. These increased rates of ethanol metabolism were observed in subjects whose nutritional status was good at the time they were drinking. This control is critical since reduction of nutritional intake may lead to the opposite effect, i.e. a decreased rate of ethanol catabolism associated with the alcohol intake.

It has been hypothesized that metabolic factors may play a key role in variations in degree of tolerance for ethanol. Fenna and his associates (3) reported that Canadian Eskimos and Indians metabolize ethanol at significantly lower rates than Caucasians. They suggested that the very high incidence of alcohol-related problems found in Eskimo and Indian populations might be due to a slow rate of ethanol metabolism in these groups. In more detailed studies, Bennion & Li (4) measured rates of ethanol metabolism in 30 American Indians and Caucasians. They used sensitive gas chromatographic techniques for assessing ethanol concentrations in blood, and they were careful to express blood alcohol data in terms of amount of ethanol metabolized per unit of body weight during fixed intervals of time. They found that the mean rate of alcohol metabolism was almost identical in the Caucasians and American Indians. In addition, these investigators examined alcohol dehydrogenase activity and isoenzyme patterns in hepatic biopsy specimens obtained from seven Indians and six Caucasians and found no differences between groups in these indices. It was concluded that tolerance to ethanol cannot be explained on the basis of differences in rates of ethanol metabolism. However, Bennion & Li (4) did find a positive correlation between rate of ethanol metabolism and past history of alcohol intake. Individuals with past histories of heavy alcohol intake had higher rates of ethanol catabolism than subjects who reported past histories of little or no alcohol intake.

The subjects studied by Bennion & Li (4) were in good health and apparently had no evidence of chronic nutritional deficiencies. Data obtained on both experimental animals (5) and human subjects (6) have indicated that nutritional intake may have an important effect on rates of ethanol metabolism. In human studies (6), systematic reduction of caloric intake during stabilized dosage of ethanol produced a marked decrease in rates of ethanol metabolism. Human studies have also revealed that decreased caloric intake by alcohol addicts during heavy drinking was not simply due to satiation produced by calories derived from alcohol. Although it might be anticipated that the caloric contribution from alcohol (which is the same as that from carbohydrate, 7 calories per gram) would decrease hunger, alcohol addicts also know that delay or suspension of eating during drinking is associated with a more pronounced and prolonged effect of ethanol. In other words, alcohol addicts learn that they can achieve a better pharmacological effect from alcohol if they do not eat. This behavior facilitates the development of serious intercurrent illnesses that are produced in part by nutritional deficiency associated with heavy drinking. Since the combination of poor nutritional intake and heavy drinking is believed to have a significant role in the genesis of a variety of disorders of the central and peripheral nervous system, the physician should attempt to convince patients who are drinking heavily to eat well and obtain diet supplements if possible. However, a patient's compliance with this recommendation may be poor because it may impair his or her need to manipulate alcohol tolerance.

Existing biochemical data cannot explain how CNS adaptation contributes to the process of tolerance. Ethanol is not metabolized in the CNS, although very small quantities of alcohol dehydrogenase have been reported to be present in some neural tissue (7). The significance of these very small concentrations of alcohol dehydrogenase remains to be determined, but it has been suggested that variation in oxidation of ethanol at cellular sites in the brain might have important effects on regional brain cell function.

It is well known that cross-tolerance occurs between ethanol and other centrally acting agents, such as barbiturates (8). Since barbiturates and ethanol are catabolized in the liver by different enzymatic pathways, it is unlikely that induction of adaptive enzymes alone could explain cross-tolerance. Some metabolic adaptation may occur because the hepatic microsomal enzyme system, which is important in barbiturate metabolism, does play some role in ethanol oxidation (9, 10). However, a complete explanation for cross-tolerance awaits discovery of some as yet unknown CNS factor.

## PHYSICAL DEPENDENCE

There has been considerable study of the significance of changes in divalent cation levels during the alcohol withdrawal syndrome. Clinical studies carried out many years ago (11, 12) showed that alcohol addicts in withdrawal had low levels of magnesium in blood, and the lowest levels were found in those individuals who had the most severe withdrawal syndrome. Subsequent studies (13) showed that treatment with parenteral magnesium might benefit patients in withdrawal, but it was also found that blood levels of magnesium rose spontaneously during remission of symptoms even when no supplemental magnesium therapy was provided. Since some alcoholics may have a total body deficit of magnesium associated with a history of poor dietary intake, and magnesium excretion by the kidney may have been increased by alcohol per se (14), the spontaneous rise in serum magnesium levels during remission of alcohol withdrawal symptoms was puzzling.

Recent studies have revealed one of the underlying mechanisms that produce fluctuations in magnesium levels during ethanol withdrawal. Wolfe and his associates (15) have observed that alcohol addicts tend to begin hyperventilating following cessation of drinking. Hyperventilation is associated with a decline in serum magnesium levels, which remain low until the respiratory rate returns to normal. During hyperventilation, there is also a significant respiratory alkalosis and degree of elevation of blood pH correlated with a decrease in plasma magnesium levels (15). It has been suggested that increased blood pH levels initiate mechanisms resulting in divalent cation movement from the intravascular pool to intracellular tissue pools, a form of cell-buffering effect. It is also thought that the decrement in plasma levels of magnesium is responsible, at least in part, for increased peripheral and CNS hyperactivity, manifest by tremor and convulsions. A significant reduction in cerebral blood flow may also occur as a concomitant of increased arterial pH. Thus, some of the central changes observed in the alcohol withdrawal state such as hallucinations, delirium, or confusion might be related to this phenomenon. The cause of hyperventilation is unknown, but it might be due to a rebound effect in central respiratory centers following depression of these centers during heavy ethanol intake.

There is also some evidence that alcohol may affect brain levels of another major divalent cation, calcium. Ross and his associates (16) have reported that ethanol administration in relatively low doses to mice produced a selective depletion of regional brain calcium. They found a significant decrease in calcium levels in hypothalamus, hippocampus, corpus striatum, and cortex 120 min after intraperitoneal injection (1.5 mg per kg) of ethanol. Morphine sulfate also produced a significant decrease in calcium content in the same regions of the brain. Calcium depletion was inhibited by naloxone, a stereospecific narcotic antagonist administered simultaneously with either morphine or ethanol. This finding lends support to the argument that a common pathway may be involved in both ethanol- and morphine-induced physical dependence.

Davis & Walsh (17) and Cohen & Collins (18) have presented indirect evidence that tetrahydroisoquinoline alkaloids may be formed in the body as a consequence of ethanol oxidation, and that such alkaloids may in turn lead to the production of physical dependence. The formation of these alkaloids is not due to the presence of ethanol per se, but to higher levels of acetaldehyde generated during the course of ethanol oxidation. Ross and his associates (16) administered salsoline, a tetrahydroisoquinoline alkaloid, to experimental animals and found that this alkaloid produced a depletion of brain calcium comparable to that produced by ethanol and morphine. Moreover, the salsoline-induced depletion of brain calcium could also be antagonized by administration of naloxone. Another hypothesis advanced to explain the related effects of ethanol, morphine, and tetrahydroisoquinoline compounds is that these may induce development of, or act as, false adrenergic transmitters. Thus it is possible that the effects of ethanol and a variety of other centrally acting drugs that can induce physical dependence may be mediated through central adrenergic neurons.

Recent advances in our understanding of the effects of alcohol on central and peripheral adrenergic neurons have been provided by the neurochemical studies of Pohorecky (19). Acute doses of ethanol in rat resulted in a decrease in norepinephrine turnover in the hypothalamus, brain stem, and telencephalon. However, an acceleration of norepinephrine turnover was found after two weeks of chronic ethanol intake. An even greater increase in norepinephrine turnover was found during ethanol withdrawal, after chronic treatment. The most marked changes in norepinephrine turnover occurred 8 hr following cessation of alcohol intake, and the brain stem was the area of the brain that showed the greatest degree of change.

It is of interest that the most marked changes in norepinephrine turnover in the rat occur at a time and in a regional portion of the CNS that is consistent with the time course and physiological effects associated with the withdrawal syndrome in humans. Observations of the time course of the alcohol abstinence syndrome in humans indicates the period of highest risk for developing tremor and seizure disorders is 8–24 hr following cessation of drinking (20–22). As noted previously, hyperventilation, respiratory alkalosis, and hypomagnesemia also have a similar time course, 8 hr following cessation of alcohol use (5). It is possible that increased norepinephrine turnover observed in the rat brain stem 8 hr following ethanol withdrawal may reflect respiratory center hyperexcitability subsequent to chronic depression of that center during long-term ethanol intake. This interpretation is speculative, since the neurochemical basis of denervation hypersensitivity and rebound hyperexcitability remains to be determined.

There is continuing controversy about the significance of adrenergic neuron sensitivity to ethanol. For example, Carmichael & Israel (23) have employed in vitro techniques to determine whether ethanol inhibits release of transmitter substances from neural tissue. The release of acetylcholine was markedly inhibited by concentrations of ethanol that produce moderate to severe intoxication in the rat. However, ethanol in pharmacologically active concentrations did not inhibit the release of norepinephrine, 5-hydroxytryptamine, dopamine, glutamate, and GABA in neural tissues studied in in vitro systems. Inhibition of release of these transmitter substances could only be produced in the in vitro system by concentrations of ethanol that would be equivalent to lethal levels in the intact organism. Carmichael & Israel (23) argue that reported changes in norepinephrine turnover in vivo produced by ethanol are not due to the action of ethanol per se, but are related to "indirect" effects.

## ALCOHOL-INDUCED CHANGES IN SEXUAL BEHAVIOR AND FUNCTION

The discordant effect of alcohol intake on enhancing sexual desire in males but inhibiting sexual function has been known for centuries. Perhaps the most succinct description of this effect was provided by Shakespeare in Act II Scene III of *Macbeth.*

> MacDuff: What three things does drink especially provoke?
> Porter: Marry, sir, nose-painting, sleep and urine. Lechery, sir, it provokes and unprovokes; it provokes the desire, but it takes away the performance: therefore much drink may be said to be an equivocator with lechery; it makes him and it mars him; it sets him on and it takes him off; it persuades him and disheartens him; makes him stand to and not stand to; in conclusion, equivocates him in a sleep, and giving him the lie, leaves him.

Feminization of male alcoholics, with or without associated liver disease, has been amply documented in the clinical literature. Van Thiel & Lester (24) have noted that feminization can occur in male alcoholics before detectable hepatic disorders. They point out that "our recent studies establish that impotence, sterility, testicular atrophy, gynecomastia and diminished body hair are prominent in chronic alcoholics whose liver function and histology run the gamut from near normal to severely deranged." These investigators postulate that excessive alcohol intake without associated liver disease may significantly effect gonadal function. There is accumulating evidence that supports this hypothesis.

In studies with alcohol addicts who served as their own controls prior to, during, and following the period of experimentally induced intoxication, we found a significant decrease in plasma testosterone levels associated with chronic ethanol intake (25). Since these patients were studied under research ward conditions, use of other drugs known to affect plasma testosterone levels could not influence these results.

However, a major limitation in this research was that plasma testosterone levels were obtained only once each day (in the early morning) and multiple determinations were not carried out during any single day. Therefore, it was not possible to correlate changes in plasma testosterone levels with hourly fluctuations in blood alcohol levels. This is an important consideration since it has been shown that hourly testosterone levels may vary widely in normal adult males. In addition, wide variation in plasma testosterone levels, unlike variations in cortisol levels, cannot be explained on the basis of large diurnal variations (26).

In more recent studies, serial samples of plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were analyzed in a group of 16 normal adult males given alcohol (27). Plasma samples were collected at 20-min intervals prior to administration of ethanol and during the ascending, peak, and descending phase of the blood ethanol curve. It was found that both plasma testosterone levels and LH levels showed significant but divergent changes as a function of blood alcohol level changes. As blood ethanol levels increased there was a decrease in plasma testosterone levels but no significant changes in levels of luteinizing hormone. At peak blood ethanol levels, a large and significant decrease in plasma testosterone levels was observed. At the same time, a significant increase in plasma luteinizing hormone levels was found. During the descending phase of the blood ethanol curve, plasma testosterone levels remained low, although higher than during the peak blood ethanol levels. Plasma luteinizing hormone levels tended to decrease during the descending limb of the blood alcohol curve when plasma testosterone levels were rising.

These data converge to suggest that alcohol intake alters plasma testosterone levels as a function of the effects of ethanol on peripheral mechanisms as suggested by Van Thiel & Lester (24). Plasma luteinizing hormone levels did not change during the ascending phase of the blood alcohol curve when testosterone levels were descending. Thus, it cannot be argued that the fall in plasma testosterone levels was due to ethanol inhibition of hypothalamic pituitary mechanisms regulating the secretion of luteinizing hormone. The increase in luteinizing hormone levels when plasma testosterone levels were quite low during peak blood alcohol levels is consistent with established data on processes of feedback regulation by testosterone of luteinizing hormone output.

The behavioral concomitants of these alterations in male sex hormones may be enhanced sexual desire, associated with CNS hypothalamic-pituitary mechanisms subserving enhanced LH output and decreased sexual performance or relative impotence as a consequence of decrements in circulating levels of testosterone. From the behavioral point of view, these hormonal changes would be in agreement with Shakespeare's statement. However, since studies that correlate levels of luteinizing hormone and testosterone with subjective feeling states relating to sexual desire and function have yet to be carried out, we can only speculate about possible relationships between sex hormone levels and behavior.

There are a number of mechanisms that may underlie the manner in which ethanol reduces plasma testosterone levels via changes in peripheral as opposed to CNS activity and metabolism. It is well known that ethanol oxidation occurs primarily in the liver and that the oxidative processes can produce alterations in the ratio of NAD/NADH. Alterations in NAD/NADH ratios significantly modify the redox state of the liver. For example, ethanol intake dramatically shifts the catabolism of catecholamines from oxidative to reductive pathways (28). Therefore, it is possible that ethanol-induced changes in hepatic metabolism significantly affect the catabolism and biotransformation of the sex steroids.

There is also evidence that ethanol may directly affect metabolic processes in the testes. Van Thiel and his associates (29) have shown that ethanol may inhibit vitamin A metabolism in the testes. They demonstrated that the oxidation of retinol by alcohol dehydrogenase in testicular homogenates was significantly inhibited by ethanol. Since vitamin A (retinol) is required for spermatogenesis, it is possible that ethanol not only acts on peripheral mechanisms, which may result in impotence, but that ethanol may contribute directly to development of sterility in male alcohol addicts via its direct effects on the testicles.

## ALCOHOL-INDUCED HEPATIC DISEASE

It is not known whether fatty metamorphosis of the liver, alcoholic hepatitis, and cirrhosis represent a continuum of alcohol-induced hepatic disorders, or whether each clinical entity derives from different and perhaps unrelated pathophysiological processes. One of the most common concomitants of heavy drinking is the development of fatty liver. This disorder is reversible and remission of fat accumulation in the liver occurs promptly following cessation of alcohol intake. However, alcoholic hepatitis is a serious disorder with mortality estimated at between 10 and 30%. Alcoholic hepatitis may also be a forerunner of cirrhosis, and cirrhosis of the liver associated with chronic alcohol abuse is a leading cause of death among males in the United States.

A key prerequisite for discovering the pathophysiological processes involved in all forms of alcohol-induced hepatic disease is the development of an animal model. A breakthrough in this area has recently been accomplished by the work of Rubin & Lieber (30). These investigators have induced a complete spectrum of alcohol-related liver disease in subhuman primates studied for periods of time ranging from nine months to four years. A crucial feature of this experiment is that ethanol was administered in the presence of a nutritionally adequate diet throughout the course of the study. The success of the animal model developed by Rubin & Lieber (30) was in part related to selection of an adequate species. The baboon has a relatively long life span, and hence ethanol could be administered chronically over intervals of months and years. All animals developed fatty livers, four animals developed alcoholic hepatitis, and two animals developed cirrhosis. The full spectrum of hepatic disease, including cirrhosis, was induced by ethanol per se and was not related to induction of nutritional deficiency. Rubin & Lieber conclude that physicians should inform patients with alcohol-related problems that "a nutritious diet will not prevent the development of alcoholic hepatitis and cirrhosis in the face of continued alcohol abuse" (30).

Fat accumulation in the liver can lead to hepatomegaly, a condition commonly observed in alcohol abusers. Baraona and her associates (31) have stressed that increases in liver mass cannot be accounted for simply by accumulation of fat. In order to determine whether substances other than fat produce hepatomegaly associated with high dosage ethanol intake, rats were fed nutritionally adequate diets along with high doses of ethanol. This regimen not only produced an increase in liver fat, but also a very significant increase in liver protein. In fact, the increase in total protein accounted for almost half of the increase in hepatic dry weight. However, the protein concentration in liver was unaltered, which indicates that water concentration increased proportionately with protein. Further studies revealed that the increase in protein could be accounted for by an accumulation of proteins that are ordinarily transported from the liver into blood. Ethanol produces an accumulation of protein as well as fat in liver, and the hepatotoxic action of ethanol may be related to accumulation of both lipid and protein. The significance of these variables for the pathogenesis of cirrhosis remains to be determined.

Not all animals given high doses of ethanol with nutritionally adequate diets develop cirrhosis, just as all human alcoholics do not develop severe hepatic disease. The factors underlying selective vulnerability remain unknown. However, there are recent data that indicate that some individuals may be at greater risk for developing alcohol-induced hyperlipidemia on the basis of a genetic predisposition. Although the relationships between hyperlipidemia, fat accumulation in the liver, and eventual development of cirrhosis are not firmly established, it is known that fatty liver usually occurs before the development of cirrhosis and that the major lipid found in the alcohol-induced fatty liver is triglyceride. In studies with alcohol addicts it was found that individuals with a Type IV hyperlipidemia showed striking elevations in triglycerides following alcohol intake, in contrast to individuals who did not have this genetic predisposition (32). Alcohol-induced elevations in triglyceride levels have also been found in normal drinkers who have a familial Type IV disorder, but have no history of alcohol abuse (33). These data indicate that social drinking can induce significant elevations in triglycerides in nonalcoholic individuals with a familial Type IV disorder.

The prevalence of Type IV disorders and their relationship to the development of cirrhosis remains to be determined. But it is generally well accepted that individuals with Type IV disorders are also prone to development of diseases such as pancreatitis. Pancreatitis, in turn, is also observed with greater frequency in individuals who abuse alcohol.

These threads of evidence offer some prospects for utilizing advances in biochemistry and genetics to reduce the incidence of alcohol problems that contribute to disability and death. For example, awareness that a genetic predisposition toward development of alcohol-induced hyperlipidemia might contribute toward a higher risk for liver disease could influence drinking behavior. At the present time, it could be argued that techniques relying on rational persuasion to abstain from behaviors that may adversely affect health are not very effective (for example, programs to dissuade cigarette smoking). But it is also important to recall that most educational techniques used for modifying alcohol consumption have not been based upon rational arguments derived from adequate data. As more adequate information becomes available concerning potential risks for developing diseases associated with alcohol use or abuse, individuals will have a better basis on which to determine their own behavior.

## ALCOHOLISM AND GENETIC INFLUENCES

It has long been known that alcoholism occurs at a higher rate among the relatives of alcoholics than in the general population (34). The interpretation of such data is greatly limited by the fact that identification of the respective contributions of learning and genetic factors has presented a virtually insurmountable methodological problem. Recently Goodwin and co-workers (35) have been able to study these questions in individuals who were separated from their biological parents soon after birth and raised by nonrelatives without subsequent contact with the biological parents. It was necessary to conduct these studies in Denmark, where there are centralized national registries of adoptions, psychiatric hospitalizations, and criminal records. Fifty-five men whose biological parent had a hospital diagnosis of alcoholism were compared to a group of 67 control adoptees matched with respect to reports of drinking problems and a variety of other psychiatric and psychosocial variables. It was found that the rate of alcoholism in the group with an alcoholic biological parent was almost four times that of the control group. The experimental group also had a divorce rate three times higher than the control group. However, diagnosable depression, anxiety, neurosis, sociopathy, and drug addiction were low and equivalent in both groups.

The drinking patterns of the adoptee parents were also studied in an effort to determine whether learning factors may have influenced the above findings. Possible or definite alcoholism was found in 13% of the adopted parents of the experimental group and 22% of the adopted parents of the control group. However, there was no significant correlation between the drinking patterns of the adoptees and the presence or absence of alcoholism in their adopted parents. The investigators concluded that children of alcoholics are more likely to have alcohol problems than children of nonalcoholics, even when they are separated from their alcoholic parents and raised by adopted parents (35).

Another study compared the incidence of alcoholism in children separated from one biological parent and raised by nonalcoholic or alcoholic foster parents. Groups consisted of children with an alcoholic biological parent who were raised by a nonalcoholic parent, and children with nonalcoholic biologic parents who were raised by an alcoholic adopted parent. The biological parent shared by the proband and by the half sibling was the mother in 54 cases. It was found that alcoholism in half siblings was associated with alcoholism in biological parents, and not with being raised by an alcoholic parent figure. Moreover, the high incidence of alcoholism in the offspring of alcoholic parents was not statistically increased by their having lived with that parent. The development of alcoholism in both full and half siblings was predicted with greater reliability by alcoholism in the biological parent than by any of the environmental factors examined. Rates of alcoholism were almost identical in children of an alcoholic biological parent who were raised by nonalcoholic parent figures and by alcoholic parent figures (36).

A similar study compared adopted sons of biological alcoholic parents with their brothers who were raised by the alcoholic biological parent (37). Both groups had comparable high rates of alcoholism (25 and 17%). Data suggest that the length of time spent with the alcoholic parent was not associated with the development of alcoholism. However, the severity of the parent's alcoholism, as indicated by number of hospitalizations, was positively related to alcoholism in the offspring. Environmental factors appeared to contribute very little to the development of alcoholism in the sons of severe alcoholics (37). These two studies (36, 37) suggest that exposure to an alcoholic parent does not necessarily increase the risk of becoming an alcoholic.

Finally, alcoholic and nonalcoholic adoptees were compared with respect to a number of childhood behaviors on the basis of their self-reports (38). Each group was interviewed concerning their childhood adjustment, school adjustment, and social attitudes as children, and subsequently about their habits, activities, and personality traits as adults.

It was found that the alcoholics were usually more hyperactive, antisocial, shy, disobedient, aggressive, friendless, and given to truancy as children. As adults, the two groups differed only with respect to drinking history, drug use, and the overt expressions of anger. Ten of the 14 alcoholics studied had biological parents who were alcoholics, whereas none of the nonalcoholic control group had alcoholic parents (38).

The extent to which hyperactivity in a social behavior during childhood predisposes adults to sociopathy and alcoholism remains to be determined, and this controversial literature has been reviewed by Goodwin and co-workers (38). There is a discrepancy between data based on retrospective historical accounts (38) and longitudinal studies, which suggest that although a high percentage of antisocial children may develop adult drinking problems, most alcoholics do not have a childhood history of antisocial behavior (39, 40). Eventual clarification of these questions awaits further research.

## CONCLUSIONS

Within the past decade, there have been a number of methodological and substantive advances that increase our understanding of the behavioral and biochemical interrelations in alcoholism. However, considerably more technological and conceptual progress will be required before the biological and behavioral bases of alcoholism are clarified.

This review has examined recent research on biochemical and behavioral aspects of tolerance and physical dependence; alcohol-induced changes in sexual behavior and function; alcohol-induced liver disease; and the contribution of genetic factors to alcohol problems. Many questions remain to be answered in each of these areas. At present it is not possible to predict which discipline will eventually contribute most to the control and prevention of this complex disorder and its numerous medical complications.

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# IMMUNOPROPHYLAXIS OF GRAM-NEGATIVE BACILLARY INFECTIONS[1]

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## INTRODUCTION

Striking changes in the pattern of infectious diseases have occurred during the last 50 years in the United States. Bacterial families, such as Enterobacteriaceae and Pseudomonadaceae, which were once considered nonpathogenic, have become the most frequent cause of hospital-acquired infections and bacteremia. Prior to 1950, bacteremia caused by gram-negative bacilli was an infrequent event, but recent reports from several large medical centers have documented a progressive increase in frequency to a current annual rate of 1 episode of gram-negative bacteremia per 100 patients in these hospitals (1–3). Although the incidence may be lower in smaller community hospitals, extrapolation of these rates to the 33,000,000 acute hospital admissions annually in the United States provides a crude estimate of the magnitude of this problem.

## EPIDEMIOLOGIC CONSIDERATIONS

Several of the factors responsible for their increasing frequency also contribute to the difficulties in controlling gram-negative bacillary infections. These include:

1. The ubiquity of gram-negative bacilli as the host's indigenous flora and in the hospital environment.
2. The propensity for antibiotic resistance which, when coupled with an environment where antibiotics are used extensively, provides a "selective reproductive advantage" to resistant strains and the selection of a "hospital flora" of resistant organisms.

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3. Hospitals provide a concentration of patients with altered host defenses who are most susceptible to infection with poorly invasive bacteria.
4. Many hospital practices allow bacteria to bypass host defense mechanisms and gain access to sites for infection (venous and bladder catheters, etc), or to deleteriously affect host defense mechanisms (corticosteroids, cytotoxic drugs, etc) (4).

In addition, several studies have shown that the severity of underlying host disease is the major determinant of outcome in bacteremia and is of such importance that it tends to obscure the effects of appropriate antimicrobial therapy (5, 6). Other studies have indicated that bactericidal antibiotics are no more efficacious than bacteriostatic agents and that if the infecting organism is susceptible, there are only minor differences in efficacy of systemic antibiotics (7). These observations suggest that new antimicrobials probably will not lower current fatality rates materially unless they exhibit differing or more effective antibacterial activity. Similarly, although a number of control measures have been shown to reduce the frequency of gram-negative bacillary infections, these have not met with ready voluntary acceptance and have yet to make a significant impact on the frequency of bacteremia.

## IMMUNOLOGIC CONSIDERATIONS

The relative limitations of antimicrobial therapy and preventive measures, as practiced, have led to consideration of alternative methods of control. The effectiveness of active and passive immunization for the prevention or treatment of other infections suggests that this might provide a feasible approach to the control of gram-negative bacillary infections. Some aspects of the epidemiology and the antigenic structure of Enterobacteriaceae, however, make immunoprophylaxis of such infections more complex than in those infections in which it has been satisfactorily utilized.

Although there is a general similarity in the structure and antigenic composition of the Enterobacteriaceae, there is also exquisite antigenic specificity of each of the three major antigens of gram-negative bacilli. Capsular antigens are present in encapsulated strains, such as *Klebsiella,* while most *Escherichia coli* possess similar structures termed "envelope" or K antigens. Motile gram-negative bacilli also possess flagellar or H antigens. The lipopolysaccharide (LPS) component of the cell wall contains the O antigen found in all gram-negative bacilli. A large number of specific serotypes of these major antigens may be found in each species. For example, 92 K, 148 O, and 51 H antigen types have been identified in strains of *Escherichia* and similar large numbers are present in *Klebsiella-Enterobacter-Serratia* and species of *Proteus* as shown in Table 1 (8). Until recently, studies in animals had indicated that only type-specific antibodies to K, capsular, and O antigens were protective in experimental infections (9–12). This large number of specific antigens among the Enterobacteriaceae would preclude any attempts at immunoprophylaxis unless only a limited number of serotypes and species were responsible for the majority of infections. The finding of 17 different capsular types among 28 *Klebsiella* and 27 different O types among 84 *E. coli* isolated from patients with bacteremia in unpub-

**Table 1** Antigenic composition of species of enterobacteriaceae

| | Number of Specific Antigens | | |
|---|---|---|---|
| Bacterial Species | Capsular or K Antigens | O Antigens | H Antigens |
| *E. coli* | 92 | 148 | 51 |
| *Klebsiella* sp. | 72 | 5 | — |
| *Enterobacter* sp. | — | 68 | 34 |
| *Serratia* sp. | uncertain | 15 | 13 |
| *Proteus* sp. | — | 117 | 44 |

lished studies from our hospital demonstrates, however, that several species and numerous serotypes of Enterobacteriaceae are responsible for gram-negative bacillary infections. The diversity of bacterial species and serotypes would make any attempt to control such infections by either active or passive immunization with type-specific antigens impractical. In contrast, clinical trials of O-specific immunization for *Pseudomonas aeruginosa* infections, where only a few serotypes are involved, are in progress (13).

An alternative approach has been suggested by recent studies that have delineated the chemical structure of the cell wall LPS of the Enterobacteriaceae. These studies originated with the derivation of a series of rough (R) mutants from smooth (S) strains of *Salmonella.* These mutants were deficient in various enzymes necessary for the sequential addition of sugars in the construction of the cell wall LPS. Delineation of the chemical structure of the LPS provided an explanation of the concomitant unique O-antigenic specificity yet similar toxic manifestations of the LPS of various gram-negative bacteria. Figure 1 presents a schematic representation of the chemical structure of the LPS of S *Salmonella* and their R derivatives, Ra through Re. The chemical structure and the specific linkages of the terminal sugars of the LPS determine the O-antigenic specificity of each organism. The inner lipid (lipid A) portion of the LPS has been shown to be responsible for its endotoxic activity. The O-specific sugars are linked to lipid A by a core portion composed of N-acetylglucosamine, glucose, galactose, heptose, and ketodeoxyoctonate (KDO) (14). The innermost portion of the core containing only KDO and lipid A, represented by the Re mutant, appears to be identical in the LPS of all Enterobacteriaceae studied to date. The composition of the remainder of the core portion of the LPS exhibits slight differences in chemical structure in various species. The chemical similarity of the core portion of the LPS of all Enterobacteriaceae suggested that there might be exposed antigenic determinants in the LPS core that were shared by most gram-negative bacilli. Studies by Galanos et al indicated that the antigenic determinants of lipid A were exposed in LPS derived from several heterologous S bacilli (15). Other studies have demonstrated that the antigenic determinant of Re mutants, distinct from lipid A, could be detected on the surface of heterologous whole S bacteria (16). These findings suggested that antibody to antigens of the core portion of LPS, which are shared by most Enterobacteriaceae, might exert protective activity against infections produced by heterologous gram-negative bacilli.

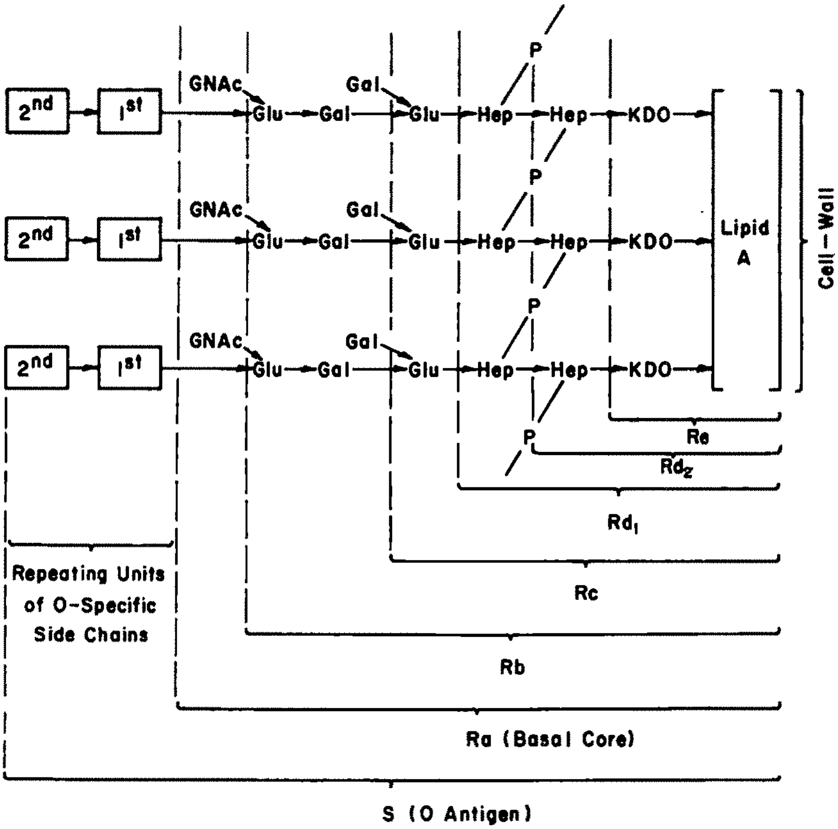

*Figure 1* Chemical structure of the cell wall lipopolysaccharide of *Salmonella.* The terminal O-specific side chains confer smoothness (S) and O-antigenic specificity. Rough (R) mutants Ra, Rb, Rc, $Rd_1$, $Rd_2$, and Re are listed in order of increasing roughness, which is produced by progressive deletions of sugars of the core structure. GNAc = N-acetylglucosamine, Glu = glucose; Gal = galactose; Hep = heptose; KDO = 2-keto-3-deoxyoctonate. (Reproduced by permission of The Williams and Wilkins Co., Baltimore, Maryland, from *J. Immunol.* 108:601, 1973).

## EXPERIMENTAL EVALUATION OF ANTIBODY TO SHARED ANTIGENS

One of the earliest indications that antibody against shared antigens of the core portion of LPS exerted protective activity was the demonstration by Chedid et al (17) that immunization with an R mutant of *Salmonella typhimurium* protected mice against challenge with *Klebsiella pneumoniae,* Type II. On the basis of these and other studies, these investigators postulated that host defense mechanisms removed S antigens and exposed R antigens, which allowed antibodies to these R determinants to protect against S bacilli (17). Although this concept has yet to be proven, other studies have confirmed that antibody to antigens of R Enterobacteriaceae protect experimental animals against endotoxins of heterologous bacilli, and both animals and man against infections with heterologous S bacilli.

Extensive investigations by Braude et al (18) showed that antiserum to R mutants and S bacilli protected against endotoxins of heterologous S gram-negative bacteria. Initial studies by this group demonstrated that passively transferred antiserum to *Salmonella abortus-equi* or an R mutant of *E. coli* O:113 produced a two- to threefold increase in the amount of smooth *E. coli* O:113 endotoxin necessary to produce an $\text{LD}_{50}$ in mice (18). Subsequent studies by this group have also demonstrated that passive transfer of antiserum to the J5 mutant, equivalent to an Rc mutant of Salmonella, of *E. coli* O:111 afforded a highly significant reduction in the frequency of both the local and generalized Shwartzman reaction (19, 20). Kim & Watson showed that active immunization with an R mutant of *S. typhimurium* induced pyrogenic tolerance to *Salmonella typhosa* endotoxin (21). Greisman demonstrated that antiserum to a glucose-deficient mutant of *S. typhimurium* and the J5 mutant of *E. coli* O:111 reduced the pyrogenic response of rabbits to heterologous S endotoxin, although they were less effective than homologous antiserum (22).

Other investigations have evaluated the protective activity of antibody to determinants of the core portion of LPS against experimental infections caused by heterologous bacilli. McCabe et al evaluated the effects of both passive and active immunization of mice with the parent smooth *Salmonella minnesota* and its various R mutants, Ra, Rb, Rc, $\text{Rd}_1$, $\text{Rd}_2$, and Re, against challenge with mouse virulent strains of *K. pneumoniae* and *Proteus morganii* (23). Both active and passive immunization with the roughest mutant (Re), whose cell wall contains only KDO and lipid A, afforded highly significant protection against challenge with both of these organisms (23). More recent unpublished studies, utilizing a granulocytopenic rabbit model of bacteremia, have demonstrated that active immunization with the Re mutant, but not lipid A, afforded significant protection against bacteremia with two different serotypes of *E. coli*. Braude et al have also shown that both active and passive immunization with the J5 mutant protected against lethal *E. coli*, *Klebsiella*, and *Pseudomonas* bacteremia in the same rabbit granulocytopenia model (24, 25).

Evaluation of the potential protective activity of antibody to the determinants of Re mutants in gram-negative bacteremia have also been carried out to insure that the protection observed in experimental animals could also be demonstrated in man. Titers of antibody to the patient's infecting organism and the shared antigen of Re mutants were measured in acute serum specimens collected from 300 patients shortly after the onset of bacteremia, and correlated with the frequency of subsequent shock and death. It was assumed that if antibody exerted a protective effect in bacteremia, high titers of antibody would be associated with less severe bacteremia and a lower frequency of shock and death than in patients with low titers of antibody. These studies indicated that high titers of IgG, but not IgM, O-specific antibodies were associated with a significant reduction in the frequency of shock and death in gram-negative bacteremia. They also provided evidence that antibody to the Re mutant afforded significant protection against shock and death in bacteremia.

Patients with high titers of antibody to Re mutants experienced shock and death only one third as often as bacteremic patients with low titers of Re antibody. This protective effect of Re antibody was independent of any protective activity of O-specific antibody (26, 27).

Two other shared antigens, common enterobacterial antigen and cell wall lipoprotein have also been described, but there is no convincing evidence that antibodies to these antigens have protective activity (28, 29).

## CONCLUSIONS

Considered together, the demonstration that a portion of the LPS molecule is shared by all Enterobacteriaceae and that antibody to these shared antigenic determinants protects animals against challenge with heterologous bacilli or endotoxin and is associated with a significant reduction in the severity of human bacteremia strongly suggests that immunoprophylaxis of gram-negative bacillary infections may ultimately prove possible. It is important to recognize, though, that any approach to the prevention or treatment of gram-negative bacillary infections utilizing shared antigens is unlikely to be as effective as type-specific immunization. Even minor enhancement of host resistance could be of considerable value, however, against bacteria with as limited invasive capacity as Enterobacteriaceae. Similarly, clinical implementation of immunoprophylaxis of this nature does have significant problems that cannot be completely described in this abbreviated review. The exact antigenic determinant of the core portion of LPS responsible for protective antibody has not been completely delineated and the development of sufficiently immunogenic but nontoxic immunizing preparations has yet to be accomplished. These appear to be feasible, but additional study will be required before necessary clinical trials of efficacy can be completed.

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# THE ADULT RESPIRATORY DISTRESS SYNDROME[1]

◆7204

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## INTRODUCTION

When respiratory failure develops as a result of intrinsic lung disease, the processes can usually be classified into two distinct clinical and pathophysiologic categories: (*a*) those involving the airways causing obstruction to airflow, and (*b*) those involving the peripheral gas exchange units (alveoli, capillaries, and intervening interstitium). An all-inclusive term, "the adult respiratory distress syndrome" (ARDS), has been applied to the latter category of disorders (1). The cardinal clinical features of the ARDS include respiratory distress, hypoxia due to intrapulmonary shunting of blood, decreased lung compliance (i.e. increased "stiffness" of the lung), and widespread infiltrations on chest roentgenograms (1, 2). These manifestations have been observed to develop in patients with a variety of underlying medical and surgical conditions (Table 1), although involvement of the lungs is often minimal or even absent in the early stages of the underlying disorder.

The validity of the term ARDS, which includes such appellations as "adult hyaline membrane disease," "shock lung," and "traumatic wet lung," has recently been debated (3, 4). The major objections to the use of ARDS is that the grouping of a variety of diverse but specific entities into a single "diagnostic" category detracts from important distinctions that should be made concerning pathogenesis, treatment, and prognosis.

The current incidence of ARDS is impossible to document. It was estimated in 1972 that there were approximately 150,000 ARDS patients per year (5). Data from two critical care units at San Francisco General Hospital (the Medical-Surgical Intensive Care Unit and the Respiratory Care Unit) show that the incidence of

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Table 1 Disorders associated with adult respiratory distress syndrome

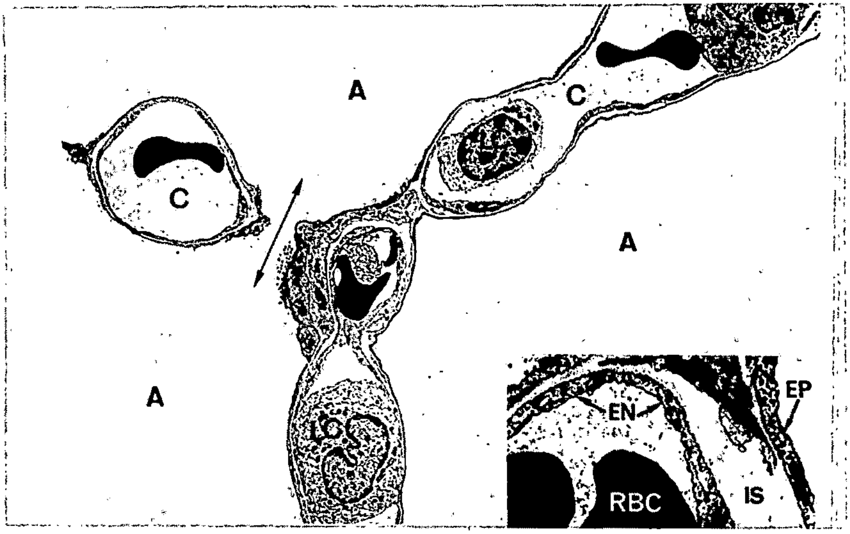
- Shock of any etiology
- Infectious causes
  - gram-negative sepsis
  - viral pneumonia
  - bacterial pneumonia
  - fungal pneumonia (rare)
  - *Pneumocystis carinii* (rare)
- Trauma
  - fat emboli
  - lung contusion
  - nonthoracic trauma
  - head injury
- Liquid aspiration
  - gastric juice
  - fresh and salt water
  - hydrocarbon fluids
- Drug overdose
  - heroin
  - methadone
  - propoxyphene
  - barbiturates
- Inhaled toxins
  - oxygen
  - smoke
  - corrosive chemicals ($NO_2$, $Cl_2$, $NH_3$, phosgene, cadmium)
- Hematologic disorders
  - intravascular coagulation
  - massive blood transfusion
  - ? postcardiopulmonary bypass
- Metabolic disorders
  - pancreatitis
  - uremia
  - paraquat ingestion
- Miscellaneous
  - lymphangitic carcinomatosis
  - increased intracranial pressure
  - eclampsia
  - postcardioversion
  - radiation pneumonitis

ARDS appears to be increasing; ARDS was present on entry to the units or developed during the patient's stay in 19 of 868 admissions in 1972, in 35 of 888 in 1973, and in 58 of 925 in 1974. The mortality rate, however, decreased from 68% to 57% to 53% in 1972, 1973, and 1974, respectively (R. Schlobohm, unpublished data). It is not known whether these figures represent an increased awareness of the condition or a true increase in incidence.

## PATHOGENESIS

### *Diffuse Alveolar-Capillary Injury*

Despite the diverse nature of the medical-surgical disorders associated with ARDS, diffuse lung injury with damage to the alveolar-capillary membrane appears to be the common denominator. This injury can be produced in a number of ways. It may be caused by infectious agents such as the influenza virus, by inhalation of toxic gases (e.g. the oxides of nitrogen), by aspiration of gastric contents containing high concentrations of hydrogen ion, or by circulating injurious agents (e.g. free fatty acids from lipolysis of fat or endotoxin). In some instances (e.g. ARDS secondary to pancreatitis), the cause of the injury is not known, and at times (e.g. ARDS secondary to shock), multiple factors may be involved. Moreover, as discussed in subsequent sections, various secondary factors, including therapeutic interventions, may aggravate the initial injury and worsen the clinical course. Regardless of the underlying cause, once diffuse alveolar-capillary injury has occurred, the ensuing sequence of events is quite similar in most patients.



**STARLING EQUATION**

$$\mathrm{F} = \mathrm{K}\left[(\mathrm{Pcap} - \mathrm{Pi.s.}) - (\pi\mathrm{cap} - \pi\mathrm{i.s.})\right]$$

$$\text{fluid flux} = \mathrm{K}\left[(10 - (-2)) - (25 - 19)\right]$$

$$\text{fluid flux} = \mathrm{K}\left[(12) - (6)\right]$$

| Permeability | Hydrostatic Pressure | Protein Osmotic Pressure |
|---|---|---|

*Figure 1.* Electron photomicrograph of a normal alveolar-capillary membrane with detail of the air-blood interface shown in the insert. *A* = alveolar airspace, *C* = capillary, *LC* = leucocyte; arrow is through pore of kohn. Insert: *EP* = alveolar epithelium, *IS* = interstitial space, *EN* = capillary endothelium, and *RBC* = red blood cell. Factors governing fluid flux at this level are related in the Starling equation. $P^{cap}$ = pulmonary capillary hydrostatic pressure, $Pi.s.$ = interstitial space hydrostatic pressure, $\pi cap$ = capillary protein osmotic pressure, and $\pi i.s.$ = interstitial space protein osmotic pressure. Photomicrograph courtesy of Drs. M. Bachofen and E. R. Weibel.

### Permeability of the Alveolar-Capillary Membrane

It is generally believed that the chief pathophysiologic consequence of diffuse injury to the interalveolar septum (Figure 1) is increased permeability of the capillary endothelium and alveolar epithelium with a leak of plasma and red blood cells into the interstitial and alveolar spaces (6, 7). The factors that govern fluid flux (*F*) across the alveolar-capillary membrane are represented in the Starling equation (8) shown in the lower portion of the figure.

Direct evidence of increased permeability has been gained by measurements of substances of large molecular weight in simultaneously collected specimens of blood and pulmonary edema fluid. Katz and co-workers (9) demonstrated in five patients with heroin-induced pulmonary edema that the concentrations of albumin and globulin in edema fluid were virtually identical to the concentrations in serum. In contrast, in five patients with cardiogenic pulmonary edema, the protein content of the lung edema fluid was only 40% of the serum concentration. Robin and associates (10) studied two patients with fulminant pulmonary edema associated with septicemia and shock and demonstrated leakage not only of plasma proteins but also of intravenously administered dextran with molecular weights up to 70,000 in one patient and 500,000 in another. Increases in pulmonary capillary endothelial permeability, alveolar epithelial permeability, or both have been implicated in the causation of experimental pulmonary edema produced by endotoxin infusion (11), *Pseudomonas* infusion (12), hemorrhagic shock (13, 14), free fatty acid (oleic acid) infusion (15), inhalation of high concentrations of oxygen (16–18), ozone inhalation (19), phosgene exposure (20), and nitrogen dioxide inhalation (21).

### Capillary Hydrostatic Pressure

Elevation of the pulmonary capillary hydrostatic pressure occurs most frequently with left ventricular failure of any etiology and results in "cardiogenic" pulmonary edema that may mimic ARDS. The major clinical differences between this form of pulmonary edema and that caused by increased vascular permeability are the severity and the poor response to treatment in permeability-related edemas (22). In patients who are severely ill with multisystem disease, the distinction between the two may be difficult to make without direct measurements of the pulmonary artery "wedge" pressure. Not infrequently, these two causes of edema may be present in the same patient, with the elevation of capillary hydrostatic pressure augmenting the tendency to edema formation produced by the increased permeability (23).

In experimental animals, neurogenic stimuli have been shown to increase capillary hydrostatic pressure (24–26). The mechanism of this increase is unclear, but may pertain to the so-called neurogenic pulmonary edema associated with seizures (27), head trauma (28), or elevated intracranial pressure of any cause (29). In addition, some investigators believe that neurogenic factors play a major role in the etiology of ARDS associated with shock (30).

### Plasma Protein Osmotic Pressure

Besides capillary hydrostatic pressure, the other intravascular force that governs fluid movement within the lung is the plasma protein osmotic pressure. Although it has been suggested that reduction of the plasma protein osmotic pressure may in itself be a cause of pulmonary edema (31), as stated by Staub (22), any reduction in serum protein osmotic pressure will be accompanied by a similar decrease in the protein osmotic pressure of the interstitial fluid; thus, no change occurs in the transmural osmotic pressure difference. Guyton & Lindsey (32) demonstrated that hypoproteinemia reduces the "threshold" hydrostatic pressure at which the accumulation of pulmonary edema fluid begins. Thus, reductions in the serum protein concentration may be clinically important and, if present, would contribute to edema formation with only modest elevations of capillary hydrostatic pressure.

### *Pulmonary Lymphatics*

Apart from the pressure relationships expressed in the Starling equation, other factors affect pulmonary edema formation. The pulmonary lymphatics serve to drain the fluid that accumulates in the interstitium of the lung. Any interference with their function will increase the water content of the lung. It is possible that relative lymphatic insufficiency may contribute to the initiation and perpetuation of ARDS. For example, it has been demonstrated in experimental animals that the normal rhythmic contractions of lymphatic vessels are absent and lymph flow is reduced while the animal is under general anesthesia (33). This situation may be analogous to that of patients who are "anesthetized" from an overdose of sedative drugs. Alterations in intrathoracic pressure relationships may also reduce lymph flows. Woolverton and co-workers (34) showed that in sheep the use of continuous positive airway pressure (see section on prevention and treatment) both reduces the flow of pulmonary lymph and significantly increases the water content of the lung.

### *Surfactant*

Accumulated evidence supports the concept of a deficiency of surfactant as one of the chief causes of the infant respiratory distress syndrome (35). Although surfactant deficiency cannot be assigned a primary role in ARDS, there is ample evidence to suggest that surfactant loss compounds the initial insult and perpetuates the pulmonary functional alterations. Normal surface activity may be impaired by one or more of the following: (*a*) removal of surfactant by pulmonary edema (36), (*b*) combination with various plasma constituents (37), (*c*) ventilation with high tidal volumes (especially from an initially low functional residual capacity) (38, 39), (*d*) effect of high concentrations of oxygen in inspired air (40), (*e*) reduction in pulmonary blood flow (41), and (*f*) systemic hypotension (42).

## PATHOLOGY

The pathologic changes are usually nonspecific (43–46). Etiologic distinctions are frequently impossible even with careful histologic examination. The early histologic features consist of interstitial and alveolar edema and hemorrhage; in addition, proteinaceous and cellular debris are present in alveoli. At a later stage, hyperplasia and organization are evident. The alveolar epithelium consists mainly of type II pneumocytes, and numerous cells are present in the interstitium (47). Hyaline membranes, thought to be the result of exudation of edema fluid containing high molecular weight substances, particularly fibrinogen, may be found lining alveolar ducts and alveoli.

M. Lamy and associates (unpublished observations) have observed significant fibrosis in biopsy specimens from lungs of patients with ARDS. We have seen significant emphysematous change develop several months after severe ARDS. It seems logical to assume that the more severe and long lasting the injury, the greater the likelihood that significant fibrosis and/or emphysema will develop.

## PATHOPHYSIOLOGY

The abnormalities of pulmonary function in patients with ARDS have been well described in a number of reviews (6, 7, 48). These disturbances fellow predictably from the previously described pathology and increase in severity as pulmonary edema accumulates and lung volume decreases. The predominant changes in severe pulmonary edema of any cause are reductions of compliance and arterial oxygen tension ($PaO_2$). The loss of compliance originates directly from increased tissue elasticity and an increase in surface forces, and indirectly from a reduction in lung volume (49). Staub and associates (50) showed that fluid accumulation in the lung proceeds stepwise to involve the pericapillary interstitium initially and then the peribronchovascular interstitium before any alveolar filling occurs. Accumulation of interstitial fluid reduces the transmural (distending) pressure across both blood vessels and airways and thus reduces their diameter (51). Because there is normally a smaller transmural pressure gradient in the more dependent lung zones (a result of the less negative pleural pressure in the dependent regions), airways in these portions of the lung are particularly prone to narrow or to close early in the course of pulmonary edema. Thus, even in the absence of alveolar edema, many alveolar units will be poorly ventilated and unable to participate fully in gas exchange. As the disorder worsens, these units become sites either of very low ventilation-perfusion ($\dot{V}_A/\dot{Q}$) ratios (i.e. markedly reduced, yet finite ventilation relative to blood flow) or of intrapulmonary shunts as documented by Wagner and associates (52). When alveolar flooding does occur, the shunt effect is augmented and gas exchange is further impaired.

King and co-workers (53) postulated that true diffusion limitation may be a third factor contributing to the hypoxia. A morphologic basis for a diffusion reduction has been demonstrated by Bachofen & Weibel (47), who showed that in addition to the marked thickening of the alveolar-capillary septum, the capillary volume per unit of tissue appears to be reduced. Assuming a normal or increased cardiac output, a reduction in capillary volume would decrease red blood cell transit time and, in the presence of an increased diffusion pathway, might reduce oxygen uptake.

In patients who survive beyond the initial edematous phase of the process, the abnormalities of gas exchange evolve so that hypoxia from right-to-left shunts becomes less important and hypercarbia with respiratory acidosis appears. This change presumably reflects the effects of the repairing process, with interstitial fibrosis and capillary obliteration resulting in an increase in the number of gas exchange units with very high $\dot{V}_A/\dot{Q}$ ratios and in a concomitant decrease in those with low $\dot{V}_A/\dot{Q}$ ratios. This shift in $\dot{V}_A/\dot{Q}$ ratios increases wasted ventilation that, unless matched by an increase in minute ventilation, results in a reduction in effective alveolar ventilation, impairs carbon dioxide elimination, and produces an increase in arterial $CO_2$ tension. Any additional reduction in lung compliance caused by fibrosis further impairs ventialtion.

## PREVENTION AND TREATMENT

In a complex entity such as ARDS, in which the etiology is multifactorial and poorly understood, specific preventive measures are difficult to define. Perhaps the key to prevention is an awareness of the situations in which lung injury is likely to develop. It is possible that prompt and vigorous treatment of the underlying medical or surgical disorder (e.g. shock) may prevent lung injury from occurring at all. More commonly, however, preventive measures are aimed at minimizing the clinical consequences that would otherwise result from a given lung injury.

### *Fluid Management*

A frequently encountered disorder in which judiciously applied therapy may prevent ARDS is shock of any etiology. Until recently, it was thought that in most patients fluid therapy for shock needed only "not be too little" and that there was little harm in overshooting the mark (54). However, this is no longer believed correct, because pulmonary edema has been precipitated by excessive infusions in patients with previously normal hearts and lungs; moreover, it follows from the Starling equation that any increase in capillary hydrostatic pressure in the face of an increase in permeability would result in an increase in water content of the lung. This effect may be inferred from the work of Brigham and associates (12), who showed that in sheep to which *Pseudomonas* bacteria were administered intravenously, causing increases in pulmonary-capillary permeability, lymph flow in the lung increased markedly when capillary hydrostatic pressure was increased slightly. Thus, a serious clinical dilemma results: overly vigorous fluid resuscitation may augment or produce pulmonary abnormalities leading to respiratory failure even in young, previously healthy patients, whereas it appears that restoration of adequate pulmonary and systemic perfusion may also be an important factor in the prevention of lung damage (55). For these reasons, it is crucial that volume replacement be carried out judiciously. In this regard, the inadequate and frequently erroneous information provided by measurements of central venous pressure needs to be emphasized (56, 57). In patients with cardiac or pulmonary abnormalities, central venous pressure does not reflect pulmonary capillary hydrostatic pressure and, in turn, does not provide a reliable guide to fluid management. The pulmonary artery "wedge" pressure employing a Swan-Ganz®[3] pulmonary artery catheter (58) is a much more useful guide. Fluid replacement and use of appropriate pressor agents can then be balanced to reinstitute and maintain critical organ perfusion with as little effect on wedge pressure as possible. Similar guidelines apply to patients who

[3] Edwards Laboratories, Santa Ana, Calif.

have not been in shock but who have undergone a process known to be associated with ARDS, such as barbiturate overdose, aspiration pneumonia, smoke inhalation, or gram-negative sepsis.

The relative effects of colloid and crystalloid solutions on the lungs have been a major source of controversy. Holcroft & Trunkey (59) have demonstrated in experimental animals that resuscitation with colloid solutions compared with crystalloid solutions may actually increase the water content of the lung; thus, use of crystalloid solutions may be preferable clinically.

### *Prevention of Atelectasis*

Because a major component of the pathology and pathophysiology of ARDS is atelectasis with loss of functional gas exchange units, the prevention of both macro- and microatelectasis may help to prevent or interrupt the tendency for air spaces to collapse. A number of factors may cause atelectasis even in normal lungs. In spontaneously breathing persons, the failure to "sigh" results in a progressive hypoxemia secondary to microatelectasis. Localized limitation of expansion related to such factors as position, pleural effusion, and ascites also results in areas of collapse. With atelectasis, there is a loss of surfactant and consequent increase in surface forces that makes reexpansion difficult. Preventive measures include mechanical ventilation with high tidal volumes (15 ml/kg body weight) and/or periodic hyperinflations, adequate removal of secretions by physical therapy and careful endotracheal suctioning when indicated (because negative pressure in the airway will cause airways to collapse, care should be taken to hyperinflate the lungs after suctioning), and frequent changes of the patient's position so no region is dependent for a protracted period of time.

### *Positive End-Expiratory Pressure*

Perhaps the most important advance in the management of this form of lung failure has been the use of positive end-expiratory pressure (PEEP). The benefit of having continuous distending pressure in the airways has been clearly demonstrated in the management of infant respiratory distress syndrome (60). Numerous reports documenting the beneficial effects of this pattern of ventilation in ARDS have appeared (61–64). From the evidence presently available, the primary effect of PEEP is to increase the functional residual capacity (65). Secondary effects that stem directly from the primary effect include (*a*) a reduction in the magnitude of intrapulmonary shunting of blood with improvement in arterial oxygen tension, and (*b*) an increase in pulmonary compliance. The latter effect is presumably the result of the increase in lung volume from recruitment of additional lung units.

Although it has been frequently suggested that PEEP may retard pulmonary edema formation, this is clearly not the case. Several studies have demonstrated that alveolar pressure either has no effect on the water content of the lung (66, 67), or may actually increase the water content by reducing lymph drainage (68). Studies in our laboratories showed that even when pulmonary function was significantly improved by PEEP, there was no reduction in pulmonary water content (69).

The use of PEEP is not without significant hazards. The increase in mean airway pressure is transmitted in part to the pleural space, thereby reducing venous inflow to the right side of the heart with subsequent reduction in cardiac output. The magnitude of this effect is the result of a balance among several factors: the circulatory status of the patient, the level of the end-expiratory pressure applied, the compliance of the lung, and the functional residual capacity at the time PEEP is applied. If a reduction in cardiac output does occur, systemic oxygen transport, the product of arterial oxygen content and cardiac output, may be reduced in spite of improvements in $PaO_2$ (70). This adverse effect of PEEP can be recognized if direct measurements of cardiac output are made. Given a constant systemic oxygen consumption, an approximation of the effect of PEEP on oxygen transport can be made by measuring mixed venous (i.e. pulmonary arterial) oxygen content ($C_{VO_2}$). A decrease in $C_{VO_2}$ indicates a reduction in oxygen transport, usually from a decrease in cardiac output. If the patient is hyper- or normovolemic, a reduction in either cardiac output or $C_{VO_2}$ indicates an excessive level of PEEP. If the patient is hypovolemic, the reduction in output may be countered by volume replacement to normovolemia as indicated by the pulmonary artery wedge pressure. In general, the more compliant the patient's lungs are, the greater will be the circulatory effects of PEEP. In patients with ARDS whose lungs are stiff, little of the end-expiratory pressure applied to the airways will be transmitted to the pleural space and the effect on cardiac output is usually slight.

Suter and co-workers (71) have devised a scheme to assess a given patient's response to PEEP that does not require measurement of cardiac output or $C_{VO_2}$. They demonstrated a good correlation between lung compliance and oxygen transport in patients with ARDS. By measuring static compliance (the volume change produced by a given static ventilator pressure) as PEEP is increased in step increments, the level of PEEP that ultimately results in a reduction in compliance can be determined. In their study, the PEEP level one step before the compliance decreased corresponded closely with the value of PEEP at which maximal oxygen transport occurred. It appears that this method offers a simple means of determining the optimum level of expiratory pressure.

The second major complication of PEEP is the occurrence of pneumothorax and/or pneumomediastinum. This presumably results from overdistention and rupture of less diseased (i.e. more distensible) alveoli. The frequency with which rupture occurs is quite variable. Pneumothorax should be anticipated in any patient receiving PEEP; in the event of a sudden and dramatic clinical deterioration, pneumothorax should be considered and a chest tube promptly placed when the diagnosis is made.

A prophylactic effect of positive end-expiratory pressure in patients who are candidates for developing ARDS has not been documented; however, experimental studies (39, 72) suggest that it may serve to prevent pulmonary edema and atelectasis in some situations. It is postulated that PEEP prevents the surfactant loss that occurs with overexpansion of alveoli from a low initial lung volume.

### *Oxygen*

In view of the hypoxemia associated with ARDS, which is often severe and life threatening, $O_2$ therapy is always required. However, the role of oxygen toxicity in producing or compounding respiratory failure has been well publicized (73). Suffice it to say that the inspired $O_2$ concentration should be high enough to provide an "adequate" $PaO_2$ (an admittedly difficult number to define) but no higher; this is generally in the 60–70 torr range. PEEP may improve oxygenation and allow a lower inspired $O_2$ concentration to be used and prevent injury to the lung.

### *Heparin*

Because microthrombi have been found in pulmonary capillaries, it has been suggested that heparin may have a preventive effect (74). This has not been rigorously examined, and, given the sorts of patients who develop ARDS, it would seem a hazardous approach in the absence of documented benefit. Even in patients with documented disseminated intravascular coagulation, the most effective approach is prompt diagnosis and treatment of the underlying cause of the coagulopathy.

### *Corticosteroids*

The usefulness of corticosteroids in patients with ARDS is highly controversial; despite claims to the contrary (75), the only accurate statement about the therapeutic value of steroids is that neither indications nor dose are definitely known. Several clinical reports purport to show the efficacy of glucocorticoids in the treatment of pulmonary fat embolism (76, 77); although perhaps valid, they are not particularly convincing. Studies of the therapeutic effect of corticosteroids in gastric juice aspiration and smoke inhalation are conflicting. It has been postulated that corticosteroids might ameliorate the long-term effects of severe lung injury and reduce the amount of fibrosis that is thought to ensue in survivors. However, because even histologically documented fibrosis may produce no significant functional alterations in at least some survivors, a drug effect would be difficult to prove.

Experimentally, at least with massive doses of methylprednisolone, beneficial hemodynamic effects are produced (78). Studies demonstrated that corticosteroid treatment improved survival in rats with experimental fat embolism (79), improved mechanics in excised rat lung exposed to high oxygen concentrations, and improved survival with less lung weight gain in rats also given a high inspired $O_2$ concentration to breathe (80).

### *Extracorporeal Oxygenation*

The use of extracorporeal membrane oxygenators (ECMO) has until now been a measure of last resort reserved for those who are obviously failing on the supportive therapy just outlined. In the largest study to date, Hill and co-workers (81) used ECMO in 25 patients with essentially end-stage acute respiratory failure. Of this group, 13 were eventually removed from ECMO, having regained sufficient pulmonary function to be managed in routine fashion. Of these 13, only four were long-term survivors. Four of the post-ECMO deaths were caused by progressive lung failure and three patients died from hemorrhage, presumably related to the anticoagulation required for the bypass procedure.

Hill and co-workers recommended the following indications for the initiation of ECMO: (*a*) rapidly deteriorating course with more than two hours of a $PaO_2 < 50$ torr while receiving maximal ventilatory support and breathing 100% $O_2$, or deterioration after 48 hours of optimum ventilatory management with $PaO_2 < 50$ torr and inspired $O_2$ concentrations $> 60\%$ for more than 12 additional hours; (*b*) possibility of reversible lung disease; and (*c*) hypoxemic myocardial or central nervous system depression. Their contraindications include: (*a*) active bleeding, (*b*) irreversible brain damage, (*c*) progressive systemic disease, and (*d*) pulmonary disease present for more than three weeks.

Using essentially these same indications and contraindications, the National Heart and Lung Institute is sponsoring a multicenter randomized, controlled study of ECMO (82). As of June 1975, 39 patients had been entered into the study. There are two survivors in the ECMO group and two in the control group. It is hoped that further results from this study will define the role of this form of treatment in ARDS.

## SEQUELLAE

The long-term sequellae seen in survivors of ARDS are poorly documented and only a few isolated serial pulmonary function studies have been reported (83, 84). The information available suggests that even in the face of what was severe respiratory impairment, pulmonary function may return to essentially normal levels. Roentgenographic and even histologic evidence of what appears to be permanent fibrotic changes have been shown to be associated with reversible functional changes. It may well be that with increasing understanding of pathophysiology and treatment, ARDS may come to be viewed in much the same light as acute renal failure with the prognosis quite good for the return of normal levels of pulmonary function.

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# ESTROGENS AND THE HUMAN MALE

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## INTRODUCTION

Although widely varying reproductive hormone concentrations characterize and are to a substantial extent responsible for the phenotypic differences between the sexes, no hormone has been described with absolute sexual specificity. While the role of androgens in the woman has been studied extensively, there has been relatively little review of evidence related to the role of estrogens in men (1, 2). The development of sensitive and specific radioligand assay procedures for circulating estrogens has resulted in a substantial recent literature clarifying the sources and disposal of estrogens and their physiological and pathophysiological roles in men. The purpose of this review is to summarize recent advances and to indicate areas for further study.

Underlying all the data to be reviewed are some fundamental principles:

1. The principal active estrogen in both men and women is 17$\beta$-estradiol ($E_2$). Estrone ($E_1$) and estriol ($E_3$), the other major circulating estrogens, probably play only a minor role under physiological circumstances.
2. The active portion of circulating $E_2$ and of testosterone (T) is the free hormone, i.e. that which is not bound to its specific plasma binding $\alpha$-globulin TEBG (testosterone-estrogen binding globulin or sex hormone binding globulin).
3. The concentration of TEBG is sex hormone dependent, undergoing induction under estrogenic stimulation and suppression as a result of androgens.
4. There is dynamic antagonism between androgens and estrogens such that the biologic effects of the one may be inhibited by the presence of the other. Although the biochemical basis of this inhibition is unknown, its influence in disease states associated with devirilizing or feminizing changes is clear.

## SOURCES OF ESTROGENS IN MEN

From shortly after birth until age 8, the serum $E_1$ and $E_2$ remains at 10 pg/ml in both boys and girls (3). In girls, values increase in relation to pubertal development and then proceed to the systematically fluctuating concentrations characteristic of the reproductive cycle. In boys, there is a gradual rise of both estrogens to a mean adult $E_1$ concentration of 62 ± 6 pg/ml and of $E_2$ to 23 ± 5 pg/ml (Table 1; 4–10).

By far the greatest portion of $E_1$ appears to be derived from the peripheral metabolism of androgenic precursors, namely T and androstenedione. In men, androstenedione is secreted at the rate of about 3 mg/24 hr by the adrenal cortex. Dexamethasone administration reduces androstenedione and $E_1$ levels parallel to cortisol (11). The importance of peripheral conversion has been established by production and metabolic clearance studies using either the continuous infusion (9) or the urinary isotope dilution techniques (1). Numerous peripheral tissues are capable of aromatization (i.e. converting T and androstenedione to estrogens), including liver, adipose tissue (12, 13), hair follicles (14), neural tissue (15), and muscle (16).

In the case of $E_2$, considerable direct testicular secretion has been established. A mean spermatic venous concentration of >1000 pg/ml was demonstrated, primarily in elderly men, compared to peripheral venous concentrations of 30 pg/ml (10, 17, 18). On the basis of $E_2$ production analyses, 75% of $E_2$ is thought to be derived from the peripheral conversion of T and androstenedione (Table 1). A rise in urinary estrogen excretion (19, 20) and increased peripheral venous estrogen concentrations (21–24) follow administration of human chorionic gonadotropin (HCG) to normal men. Such data do not establish whether the estrogen increment represents direct testicular secretion or peripheral conversion of secreted T. Proof of gonadotropin-dependent testicular estrogen secretion is provided by the demonstration of increased spermatic to peripheral venous $E_1$ and $E_2$ gradients following HCG administration to normal men (5).

Each of the cellular elements of the testis has been proposed as a site of estrogen synthesis. Direct evidence for aromatization of androgens by both interstitial (25) and Sertoli cells (26) has recently been shown.

Table 1 Sources of estrogens in normal man

| Hormone | Estrone | 17 β-Estradiol |
|---|---|---|
| Plasma concentration (pg/ml) | 62 ± 6.6 (4, 5) | 23 ± 1.6 (4, 5) |
| Metabolic clearance rate (L/24 hr/$m^2$) | 1170 ± 70 (6) | 820 ± 40 (6) |
| Blood production rate (μg/24 hr) | 45–80 (1, 7, 8) | 45 (1, 9) |
| Major precursors | Δ4[b] | T[c], Δ4 |
| Precursor secretion rate (μg/24 hr) | 3000 | T = 7000<br>Δ4 = 3000 |
| $\rho$ (Transfer constant)[a] | 0.0135 (9) | $T \to E_2$, 0.004 (9)<br>$\Delta 4 \to E_2$, 0.07 (9) |
| Percentage derived | | |
| precursor conversion | > 95 (1) | 80–100 (1) |
| testicular secretion | < 5 (5) | 10–25 (10) |

[a] $\rho$ = Percentage of infused precursor converted to product.

[b] Δ4 = Δ4-androstenedione.

[c] T = testosterone.

In the male, then, estrogen production is due largely to peripheral conversion of secreted androgens, so that the serum level of $E_2$ is determined in the first instance by those mechanisms that regulate androgen secretion. At least two additional control points are known, which may be of considerably greater importance in determining the estrogen-androgen dynamic balance: conversion rate and hormone binding (Table 2). Siiteri & MacDonald have demonstrated a linear increase in the rate of conversion of androstenedione to $E_1$ with age and increasing adiposity, and possibly with onset of puberty (1). These data imply that both the production of hormone precursors and the rate of conversion to active products exert a powerful impact on net hormonal balance. Analogous control points have been demonstrated recently for the interconversions of thyroxine to triiodothyronine (27) and 25-hydroxycholecalciferol to 1,25 dihydroxycholecalciferol (28).

Estrogens and androgens are the most potent physiological regulators of the secretion of the specific $\alpha$-globulin in serum that binds $E_2$ and T (termed both SHBG and TEBG). TEBG normally binds all 17-$\beta$ hydroxylated $C_{18}$ or $C_{19}$ steroids. It has a single binding site with a very high affinity for 5$\alpha$-dihydrotestosterone, 5$\alpha$-androstenediol, T, and $E_2$ (29). TEBG concentration is increased as a result of estrogen administration and pregnancy (30–34), hyperthyroidism (30, 35, 36), and, in men, with aging (37–40), and is decreased by androgens and progestins (34). Under normal circumstances, TEBG binding capacity ($3 \times 10^{-8}$ M) exceeds the serum T ($2 \times 10^{-8}$ M), but the binding capacity may readily be exceeded. By contrast, $E_2$ with a serum concentration of $10^{-10}$ M is always far from saturation.

Only that small fraction of $E_2$ or T which is not TEBG bound is biologically active (36). Since T binds to TEBG with greater affinity than $E_2$ and is much nearer saturation, a change in binding globulin concentration exerts a more profound effect on free T than on free $E_2$. Consequently, the increase in TEBG concentration attending a small increment in $E_2$ may amplify the effect of the estrogen by reducing the free T to a much greater degree than the free $E_2$. Androgen administration would have a converse effect (41).

## ESTROGEN METABOLISM

An enormous literature has accumulated regarding estrogen metabolism, and the interested reader should consult the review by Adlercreutz (42). A wide variety of

**Table 2** Regulatory controls over reproductive hormone action

1. Gonadal secretion rates, dependent upon gonadotropin levels and intrinsic secretory capacity
2. Production from precursor steroids, dependent upon precursor secretion rates and proportion of metabolism by a specific pathway (transfer constant)
3. Concentration of binding proteins (TEBG)
4. Rate of interconversion of active hormones
5. Rate of irreversible metabolism of active hormones
6. Integrity of the intracellular binding translocation and transcriptional control system for differentiated cell function

steroid transformations is carried out by the human liver. $E_2$ is transformed largely to $E_1$ and to estriol ($E_3$). In addition, 16-hydroxylation, 2-hydroxylation, and 2-methylation take place. The estrogens are also conjugated to glucuronides and sulfates, which are then excreted through biliary and renal mechanisms. There is indirect evidence that a small amount of androstenedione is converted directly to estrone sulfate in peripheral tissues and circulates as free estrone only after deconjugation (43–46).

## THE PHYSIOLOGICAL ROLE OF ESTROGENS

Recent advances in molecular endocrinology have refined the definition of a hormonal target tissue as one possessing a specific high affinity receptor or binding protein for the hormone. Such receptors for $E_2$ have been found in the testis, prostate, pituitary, hypothalamus, and adrenals of the male rat, and in hypothalami and pituitaries from the male human fetuses (47, 48).

In men, pharmacological doses of estrogens, principally used for the treatment of prostatic carcinoma, produce gynecomastia, decreased libido, softening and thinning of the skin, decreased facial hair, redistributed body fat, increased hormone binding globulins, and significant increases in body weight, blood volume, and blood pressure (49–51). A concomitant increase in mortality due to coronary heart disease and the consequences of decreased libido have provoked a substantial reduction in the therapeutic dose of estrogens in the management of prostatic cancer (52).

It has not been possible to establish a specific physiological role for circulating estrogens in men. Prolonged administration of relatively small amounts of potent estrogens will result in diminished T secretion and reduction of seminal fluid and sperm production, attesting to their potency as gonadotropin inhibitors (53, 54) and perhaps as direct inhibitors of testicular function (55). Other relevant influences on the hypothalamic-pituitary axis include a specific inhibition of pituitary responsiveness to GRH (gonadotropin releasing hormone) (56, 57). It has even been suggested that androgens act at the hypothalamus only through local conversion to estrogens (58), but that concept is refuted by the demonstration that nonaromatizable androgens inhibit gonadotropin secretion (59). A sophisticated frequent sampling experiment has shown a disparity between the hypothalamic-pituitary effects of androgens, which reduce the frequency, and estrogens, which reduce the amplitude of gonadotropin discharges (60), supporting the idea that the two hormone groups exert different effects on the same target tissues.

## PATHOPHYSIOLOGY

The astute observation of Dr. William Bean that many of the cutaneous manifestations associated with advanced hepatic cirrhosis resemble those of pregnancy led him to postulate an estrogen excess state in cirrhotic men (61). Furthermore, since gynecomastia commonly accompanies estrogen administration, those conditions resulting in gynecomastia have been lumped into an estrogen excess group. In no case is the information complete. The best data suggest that an increase in the free

$E_2$:T, rather than the absolute value of any single hormone, is the sine qua non of estrogen excess.

Conditions that have been associated with gynecomastia are presented in Table 3.

## PUBERTY

During the course of puberty, a degree of gynecomastia can be found in over 60% of boys (62). Occasionally, it progresses rather than resorbs, and requires excision. While no hormonal mechanism has been established, it has been suggested that early in puberty, prior to a major elevation in T concentration, there is an increase in the conversion of androstenedione to $E_1$ and possibly to $E_2$ (1).

## CIRRHOSIS

Hepatic cirrhosis may be associated with gynecomastia, testicular atrophy, spider angiomata, and palmar erythema. A number of hormonal abnormalities have been demonstrated. There is a delay in the metabolic clearance of $E_2$ (63), due in part to a marked increase in TEBG concentration (64, 65). T levels are reduced, usually accompanied by normal $E_2$ concentrations (64–66); elevated $E_2$ levels have been observed in some patients (67, 68). Testicular responses to administered gonadotro-

**Table 3** Conditions associated with gynecomastia

- Puberty
- Metabolic disorders
  - hepatic cirrhosis
  - thyrotoxicosis
  - renal failure
  - refeeding
- Drugs
  - estrogens, androgens
  - digitalis
  - spironolactone
  - tranquilizers
- Hypergonadotropic hypogonadism
  - Klinefelter's syndrome
  - male pseudohermaphroditism, Type 1, including Reifenstein's syndrome and testicular feminization
- Tumors
  - adrenal cortical carcinoma (occasionally)
  - gonadotropin producing tumors of reproductive tissues (testis) and nonreproductive tissues (lung, liver)
- Local conditions of the chest wall
- Idiopathic

pin and pituitary responses to clomiphene citrate stimulation are also impaired in a significant number of patients (64, 65). The most impressive change is a substantial increase in conversion of androstenedione to T, $E_1$, and $E_2$ (69).

The physiological consequence of these changes is a large increase in the free $E_2$:T . The reason for the rise in TEBG is unknown, although hepatic synthesis of the protein is induced by estrogens and inhibited by androgens. It also may be due to a general increase in globulin synthesis to compensate for reduced albumin production.

There is no information about the mechanism by which increased conversion of androstenedione to active androgens and estrogens is induced.

Attending the abnormalities outlined above is an impairment in estrogen metabolism: biliary excretion of estrogens is decreased in both acute and chronic liver disease. Whether this results from delayed uptake, enzymatic transformation, or biliary transport is not certain, but it is accompanied by decreased 2- and 16-hydroxylation, increased urinary estrogen excretion, and in severe disease, increased urinary free estrogens (42).

## HYPERTHYROIDISM

Histological evidence of gynecomastia has been observed in 83% of a series of hyperthyroid men (70). Studies in spontaneous and induced hyperthyroidism in men show a seven- to eightfold increase in TEBG concentration, not accompanied by increases in other hormone binding properties, such as corticosteroid binding globulin (36, 71). Consequently, there is a decrease in the fraction of unbound T (72) and compensatory elevation of LH (72, 73), followed by restoration of the plasma unbound T to normal (72). Furthermore, accelerated production and aromatization of androstenedione both contribute to an increase in free and total $E_2$ (72–74), suggesting that excessive estrogens may underlie the increased TEBG concentration. As a result of the above changes, the free $E_2$:T ratio is elevated (72).

## CHRONIC RENAL FAILURE

In chronic renal failure, gynecomastia has been noted, primarily but not exclusively related to the institution of hemodialysis (75–79). In some cases, it may resemble "refeeding gynecomastia." In chronic hemodialysis, total serum $E_2$ and T have been reported to be normal, but gonadotropin levels are very high (78, 79). The etiology of gynecomastia in this condition has not been established. The fact that many of these patients receive androgenic and anabolic steroids complicates the situation.

## REFEEDING

Refeeding gynecomastia has been described in association with successful treatment of tuberculosis and upon recovery from the deprivations of concentration camp existence (80). Although there is no direct evidence, studies of patients recovering from anorexia nervosa suggest that protein starvation results in regression of the hypothalamic-pituitary axis to a prepubertal state, which is then passed through once more upon refeeding (81). This would suggest that the mechanism of gynecomastia in these cases is similar to that for puberty.

In a recent study, men with protein-calorie malnutrition demonstrated clinical hypogonadism, low total and unbound T, and low total $E_2$. LH was elevated in most patients. These parameters reverted toward normal with refeeding. It is of interest that the one patient who developed gynecomastia showed a doubling in unbound $E_2$ during the refeeding period (82).

## DRUG ADMINISTRATION

Gynecomastia has been a frequently recognized consequence of digitalis administration (83), and has been attributed to estrogen-like activity of the glycoside (84). A recent study has shown increased total serum estrogens and decreased plasma testosterone in men treated with digoxin (85). The mechanism remains unknown, and it is not clear whether the abnormalities reflect the effects of heart failure or those of the drug itself. The effects of spironolactone are less ambiguous. This diuretic causes a substantial incidence of gynecomastia when used in high dosage. Recent studies show that the drug interferes with T biosynthesis. Conversion of androstenedione and T to $E_1$ and $E_2$ remain normal, so the drug would be predicted to cause a reduction in the relative concentration of free T (86). Other drugs associated with gynecomastia, e.g. phenothiazines and amphetamines, have not been completely studied, but are known to result in increased serum prolactin levels. Adequate information regarding the effects of these agents on the concentration of gonadal steroids is not available.

## NEOPLASMS

Gynecomastia may accompany a diverse group of tumors, including bronchial, testicular, and hepatocellular carcinoma, and may precede other evidence of tumor growth by many months (87, 88). The common feature of these tumors is elaboration of an LH-like gonadotropin. The consequences of gonadotropin excess regarding steroid secretion and interconversion, and plasma concentration are not known. Some patients with germinal epithelial tumors have been reported to have increased urinary estrogen excretion (89–91). Elevation in plasma $E_2$ has been documented in a patient with choriocarcinoma and gynecomastia (92). The source of estrogen in these patients remains obscure.

Less commonly, feminizing adrenocortical carcinoma is encountered. Uniformly, among 52 cases reported, urinary estrogen excretion was increased (93). Estrogen production has been demonstrated in an adrenocortical tumor in vitro (94), suggesting that direct estrogen secretion may sometimes be involved. However, the large amounts of androgenic precursors such as androstenedione, which are typically secreted by functioning adrenocortical carcinomas (95), must also contribute substantially to estrogen production in many cases.

Estrogens have not been implicated directly in the epidemiology of any tumor in the human male. Some evidence for abnormal estrogen metabolism (96) and for increased urinary estrogen excretion (97) has been presented for men with carcinoma of the breast, but circulating levels and production rates of estrogen are unknown.

## HYPERGONADOTROPIC HYPOGONADISM

By far the most common hypogonadal state presenting with gynecomastia is Klinefelter's syndrome, where severe seminiferous tubular failure and hyalinization is accompanied by a relative deficiency in basal T secretion and a reduced response to HCG (98). There is some evidence of a relative increase of estrogen vs androgen production (99, 100). In the one study in which serum $E_2$ values were reported, they were elevated (101). Data relating to the remaining hypogonadotropic hypogonadal states is fragmentary.

Testicular feminization, the most extreme form of male pseudohermaphroditism, has been shown to be due to diminished responsiveness of tissues to androgens secreted during fetal and postnatal life (102). Despite an XY chromosome complement and normal or high T concentrations, there is development of the female phenotype. The female phenotype results from the absence of androgen effect during embryonic development. Moreover, maturation of the female secondary sexual characteristics at puberty is due to substantial estrogen production (103, 104), half of which derives from testicular secretion (104). In the absence of the inhibitory effects of androgens, rather complete feminization is the rule.

Incomplete male pseudohermaphroditism, Type 1, is a variant of testicular feminization. It is typified by the Reinfenstein syndrome and other cases showing varying degrees of genital ambiguity. The common defect is androgen resistance, most probably due to an abnormal target tissue receptor mechanism. Estrogen production is excessive, and results largely from testicular secretion secondary to reduced negative feedback sensitivity in the hypothalamus and its attendant increase in LH concentration (104). This should be distinguished from the syndrome of incomplete male pseudohermaphroditism, Type 2, in which gynecomastia does not develop. These patients manifest normal estrogen production and LH concentrations. Apparently, those tissues requiring conversion of T to dihydrotestosterone antenatally—the urogenital sinus and tubercle—fail to develop, resulting in a female phenotype with partial masculinization at puberty (105).

## AGING

A significant number of men experience a decline in testicular function with advancing age. The mean serum concentration of T is decreased in men over 65 years (38, 40, 106), and is associated with significant elevations of LH, FSH, and TEBG, with reduction in the apparent free T concentration (38, 106). Total serum $E_1$ and $E_2$ are elevated, and although the apparent free $E_2$ remains normal, the ratio of free $E_2$:T is increased (106). Although the increase in LH suggests a testicular source for the estrogen, it more likely derives from peripheral conversion of secreted androgens, since accelerated aromatization of androgens has been found to increase with age in men (1).

The significance of these findings is not clear. The decrease in mean serum T is accompanied by an increase in the range of T concentrations (40). Thus, it may be possible to distinguish a hypogonadal subgroup within the aging male population. The relationship of increased estrogen production to the development of hypogonadism is of major interest, but remains obscure.

## RELATIONSHIP OF ANDROGENS, ESTROGENS, AND BEHAVIOR IN MEN

A great deal of attention has centered on the complex interrelationships of sexual behavior, aggression, and endocrine status. Thus, several investigators have indicated a high correlation between indexes of aggression and serum T in normal and institutionalized men (107, 108). In addition, psychological stress results in suppression of the serum T concentration (109). Of great interest is the possible relationship of circulating T to sexual behavior itself. Removal of effective T levels by castration, or by administration of the antiandrogen cyproterone acetate results in marked reduction in libido. Such therapy has, in fact, provided the basis for effective treatment of habitual sexual offenders (110). Of particular concern is the possible role of androgen in the development of gender preference. A significant decrease in serum T has been observed in a group of homosexual men, with an inverse correlation between the exclusivity of homosexual preference and T concentration (111). In addition, plasma LH was elevated in the homosexual group (112). Confirmation of these results has been difficult to obtain. Two separate studies of adult men and one study of adolescents did not show differences in T concentration between homo- and heterosexuals (113–115). In one large group of homosexuals, T levels were normal, but a significant increase in $E_2$ was found (116). The reasons for these discrepancies are unclear at present, although analysis of free T and $E_2$ and consideration of the free $E_2$:T ratios may resolve the issue. Even if altered hormone levels are shown to be a consistent feature of a subgroup of homosexual men, there is no evidence to support a causal inference about such an abnormality. Furthermore, there has been no evidence presented to indicate an alteration in overall libido in these individuals.

## CONCLUSIONS

Review of the current status of the sources and physiological effects of estrogens in the male has led to important questions for further study.

1. What are the mechanisms by which estrogens and androgens exert antagonistic effects on the same tissues? They do not compete for the same receptors.
2. The fractional conversion rate of androgens to estrogens is not constant, but varies greatly with adiposity, age, and other conditions. The loci of peripheral metabolism and the controls over the rate of aromatization are not understood.
3. Pubertal gynecomastia has no established etiology. The rate of aromatization of androgens, testicular $E_2$ secretion, and influences of prolactin secretion have not

been elucidated. This is also true in the case of gonadotropin producing neoplasms in which initial testicular function is presumed normal.

4. The role of the free $E_2$:T ratio in human male social and sexual behavior has not been subject to intensive investigation. Although a few studies have addressed the question of a role for reproductive hormone abnormalities in deviant sexual behavior, little is actually known.
5. The interrelation between behavior, nutrition, hormone secretion, and degenerative changes, such as benign prostatic hyperplasia, will be an important topic of study.

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# PSYCHOLOGICAL ASSISTANCE FOR THE DYING PATIENT AND HIS FAMILY

❖7206

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Long the poor stepchild of both medicine and psychiatry, the study of dying has taken on new life and has even been dignified with a new name. Until this decade the term *thanatology* could be found only in an unabridged dictionary. Now there are departments of thanatology, a society of thanatology, and an ever-growing number of professionals who identify themselves as thanatologists. Most thanatologists are psychologists, social workers, social scientists, clergymen, and nurses. They are not especially well disposed toward physicians.

Doctors, since the advent of modern medicine, have been far more concerned with curing than with caring for the incurable. This focus has yielded obvious benefits for the patients who can now be cured, but the concomitant negligence with respect to the process of dying and its problems cannot be overlooked. Many physicians have attempted to excuse their negligence by accepting as fact many myths about the dying patient. Examples are easy to find: "People do not want to be told they are dying. Such information can only depress the patient." "My duty stops with telling the next of kin." "I believe the job of telling and comforting belongs to the clergy." "To tell the person he is dying is to deprive him of hope."

There is some truth in each of these statements. They appear logical. They possess an enormous appeal for the harried physician because they rationalize the natural tendency of the doctor to avoid being a messenger of despair. If such myths are questioned, the doctor's life becomes gravely complicated. He has no training to prepare him to help a person die.

Until very recently, no courses on dying were offered in medical schools and no provision for dealing with this subject was included in any program for training residents. Doctors, who see more of death than anyone save the undertakers, have been taught how to pronounce the patient dead, but not how to ease the psychological distress of dying.

Until 1959 there were few studies on the psychology of the dying patient. Sir William Osler was one of the few internists who showed an interest in the process of dying (1). His statement that dying patients rarely appear to be in physical or mental pain is often quoted to justify the doctor's laissez-faire policy. The first publication on dying to receive a wide audience was by a psychologist, Herman Feifel (2). Shortly thereafter other authors, many of whom were physicians, began publishing their observations.

It is noteworthy that the findings of these investigators were all in agreement on four crucial issues (3–6). To my knowledge, no study refutes the four basic findings of this early research. These are:

1. Despite what has been told and what withheld, most dying patients know the truth about their illness.
2. Familiarity with the unpleasant facts of a terminal illness need not destroy hope and breed despair.
3. Fear of death itself is seldom expressed. It is fear of the process of dying that produces distress.
4. Most patients do not ask directly about their prognosis. They do so indirectly.

As can be seen, these points all hinge on communication. They revolve about whether or not to be open and truthful. While this may not be as important a factor now, it must be remembered that as recently as 1961 a poll of physicians showed that 93% favored not giving the patient the full facts about his illness (7). A similar survey taken now would probably reduce the 93% to 50%. It is not necessary for a physician to spell out in detail the diagnosis and prognosis to the patient at one sitting. It is crucial to show a willingness to listen, to answer questions as they occur, and to exchange vital information with another human being. A good doctor-patient relationship is forged with bonds of mutual trust and understanding.

Although it came as no surprise to those of us who work with dying patients, the early data of Feifel was met with cynical disbelief by many physicians. Feifel found that approximately 89% of his dying patients knew their diagnosis and their prognosis even though they had not been told. This information is not difficult to come by if one considers the changes that occur in the management of patients who are considered terminal. Generally speaking, they are visited less frequently by their physicians, their narcotic and sedative intake is increased, the number of diagnostic procedures falls off, and they are less often encouraged to participate in activity programs (i.e. occupational therapy). This is exemplified by the following: A woman with a terminal breast cancer asked her doctor why her headache persisted. When the doctor said it was probably nerves, she asked why she was nervous. He returned the question. She replied, "I am nervous because all the tests have stopped, nobody wants my blood, and I get all the pills I want. The priest comes to see me twice a week, which he never did before, and my mother-in-law is nicer to me even though I am meaner to her. Wouldn't this make you nervous?" There was a pause. Then the doctor said, "You mean, you think you are dying?" She said, "I do." He replied, "You are." Then she smiled and said, "Well, I broke the sound barrier; someone finally told me the truth." The truth had been announced to this woman as emphatically by voiceless signs as it would have been through a megaphone.

It is well to remember that keeping a secret involves at least two people. It is an interpersonal issue. The more individuals included in the secret, the more likely one is to betray the truth. Since the group of people around the dying patient is often large, it is impossible to insure a conspiracy of silence. This being the case, the physician should make it his policy to be as open as possible, ready to answer all questions even if they only hint at concern with the issue of mortality.

Giving the patient the truth about his diagnosis and prognosis need not destroy hope and induce severe depression. The human being is far more capable of responding with strength and fortitude to adversity then we think. To predict that the gloomy facts of a prognosis will obliterate hope is to forget the capacity of the human to endure and persevere in extreme hardship. Such a forecast is an expression of the forecaster's projected fear rather than a true assessment of the situation. How often the healthy mistake the psychology of the sick by unconditionally identifying with them. All of us in medicine must realize that illness affects people in a variety of ways. Our own set of imagined responses may be totally incongruent with those from the sickbed. Furthermore, it is not so much the truth that counts, but the way it is told. A proposed plan of action takes some of the sting from bad news. To tell a patient he has a spreading and inoperable malignancy and leave it at that is to tell him he has reached the end of the road. Radiation and chemotherapy should be offered as desirable choices when appropriate. No matter how extensive the metastases or the degree of malignancy, the patient wants to know that he is in the hands of someone who will not give up fighting the lesion itself until the time arrives when active measures are no longer possible. Then the patient should rest assured that his doctor will still be standing by to make him as comfortable as possible. The main principle here is that information should be given in tandem with hope. Hope can often be provided to the sickest patient by the simple measure of setting down a program of therapy.

There is a study from Scandinavia that demonstrates the practical value of truthful management (8). At the University of Lund, 101 patients suffering from inoperable cancer were divided into two groups. One half were told all the facts of their illness, the other half told nothing. During the year of the study it was found that those not told had more anxiety and depression and used more drugs. Their overall management was more difficult than for those patients who were told. Although this study is methodologically faulty it does underscore what a number of clinicians have long suspected.

Dr. Kubler-Ross describes five stages through which a patient may pass upon learning his prognosis (6). We look upon them less as stages than as responses that may occur in sequence as Dr. Ross described or may occur and recur throughout the entire course of an illness. The first stage is that of denial and isolation. The patient does not believe the facts as they are told him. He will, for example, think a mistake has been made in the biopsy report. He may regard himself as a special case exempt from the usual course of cancer or he may simply refuse to believe that cancer is in his body. The second stage is that of anger. The patient is angry at doctors, angry at family, angry at God or fate. This is followed by a state of depression in which the individual withdraws, loses interest, and feels doomed. It is followed by the fourth stage, called bargaining. He bargains with God or with his own physiology, exchanging something for extension of time. This is followed by the stage of acceptance, which is really more a goal to attain than a permanent state of mind. Acceptance is described as the state in which the patient is neither depressed nor angry about his fate. It may be accompanied by or caused by physical weakness. It does not mean that happiness replaces anguish. Often the equanimity is short-lived and can be disrupted by memories from the past and fears about what is to come. Acceptance is most accurately described by calling it a state of resignation where there is little fight and little of the anguish of despair.

The state of mind and mood of the dying is one of constant change. Assuming that he has been told the full truth, one would expect more periods of gloom and foreboding than of participation and pleasure. There seems to be a buoyancy in the human spirit, an ability to persist and thrive even in the bleakest of times that comes to the aid of the dying. Areas of perception can be walled off. The cognitive process can be slowed or altered so that reality is tilted in favor of the patient. These ups and downs are referred to by some as middle knowledge. This refers to a state of uncertain certainty. The background is that of certainty. If called upon the patient could say that he is dying. However, at times his hope flourishes and his pessimism pauses. It should not give the physician concern if he sees this occur in his patients. Middle knowledge is the result of two opposing forces—the reality of the situation as told to the patient and perceived by him, and his intense desire to negate the dreaded information. Some days these forces will be in balance. Other days one will be ascendant. As a consequence, affect may change from day to day. Some days the quotient of foreboding may be reduced by a new hope. The visitor will then find the patient confident and optimistic despite his cachectic appearance. An hour later he may be overwhelmed by despair at the appearance of a new pain that has dissolved all hope in an anguish of recognized truths.

The finding that the fear of death (personal extinction) is less important than the fear of dying is not so much a play on words as it may seem. The paradox of death is that we cannot imagine our own death even while certain that it will overtake us. On the other hand, the process of dying entails a number of unpleasant things such as pain, a multitude of iatrogenic discomforts, and separation from loved ones. A fear far more commonly voiced by the dying than fear of the grave is fear of lonely suffering. When reassured through word and deed that abandonment will not occur, life becomes easier.

Another point investigators agree upon is that most patients do not ask directly about their diagnosis or their prognosis. This seems to be especially true in the blue-collar class. As the level of education increases, so does the patient's ability or willingness to question his doctor. This is an important point for those physicians who say, "I only tell the truth to patients who ask me." Using this shibboleth, the physician would rarely be required to speak out. The patient obliquely questions his prognosis by talking about the future or making plans. For example, a 72-year-old man had widespread metastatic illness. Everyone believed he would be dead within three weeks. His physician adamantly refused to discuss death. The patient began to make plans for selling his house and moving to an apartment in town. These plans were allowed to continue and came to involve a considerable amount of money. In fact, the patient was either deliberately or unconsciously challenging the physician to speak up by proceeding with these costly and futile plans. It was only just before the title changed hands that the physician allowed the psychiatrist to tell the gentleman the story. He was not surprised and muttered, "In matters of money and property the truth will out."

The important message in all of the major studies cited is that it is possible to talk with dying patients. Patients enjoy and profit from such exchange. Once we make it known that we are willing and open to discussion, everyone feels more comfortable. Not infrequently, the patient expresses gratitude for the chance to discuss vital aspects of his illness that had been kept under cover in the presence of family and physician. In my experience, which covers contact with over 500 cancer patients, only a small number preferred not to talk about their illness or the future. The others welcomed the opportunity.

Families, in their response to imminent loss, fall easy prey to confusion and misplaced altruism. In an attempt to lighten the burden of the dying, they often want the truth withheld. This only heightens the patient's sense of isolation and loneliness by diminishing communication. Again utilizing the lesson learned from the investigations of the last decade, it has repeatedly been found that when communication falls off, depression, loneliness, and despondency flourish. If the communication in the family has been open and full in the past, it will probably be maintained during the course of a terminal illness. In families where communication has been obfuscated by lies, evasions, and half-truths, these maladaptive patterns are apt to continue unabated while a member dies. The physician must ensure that faulty communication does not add to the patient's burden. Families often, if not always, need guidance about what can and should be said. Once the guidelines are established and agreed upon they are usually able to manage on their own. They need to be told the importance of truth, of answering questions in a forthright way, and of not dodging issues. The families of children who are dying require far more counseling and support than do those of adults. Generally speaking, the families of dying children should have the benefit of professional counseling.

Physicians often turn to the clergy for help in the management of the dying patient and his family. While this is good general policy, it is a misconception that all clergy are skilled in this role. Unless he has had special training in a chaplain's program, the priest, minister, or rabbi is in no better position to help the dying than the average M.D. Unless the physician knows the clergyman to be effective in the care of the dying, it could be a mistake to depend upon him for handling the emotional needs of the patient and his family. This is not to say the clergy cannot be extremely helpful; there are innumerable instances where they have been. However, it is important to realize that clergymen are not specifically trained in the care of the dying and sometimes resort to a ritual of prayer just as the physician and the nurse resort to their ritual of pulse taking, auscultation, and the examination of well-healed wounds.

The reader may now ask: Having told the patient the truth, what is the next step? One can speak about the treatment of the dying, but what are the components of this treatment? There are five common complaints of the dying. These are pain, dyspnea, nausea, loneliness, and fear of suffering. Treatment begins by realizing one's limitations in terms of the last two fears—loneliness and fear of suffering. It is the natural tendency of physicians, when their patients are no longer curable, to turn their energies to those with illnesses amenable to their skill. As a consequence doctors tend to visit dying patients less frequently and to spend less time in the sickroom with them. The first step in the proper treatment of the dying is to maintain the same number of visits and to prevent this pattern from being disrupted no matter how persuasive the reason. My next suggestion may seem unnecessary, patronizing, or simple-minded, but I make it without apology. While visiting the patient, sit down. Doctors have the distressing habit of standing at the foot of the bed poised like a Nike missile ready to depart. Their entrance is nothing more than a countdown for takeoff. If one sits down, he need stay no more than a minute or two, but in that minute the fact that he is sitting at eye level with his patient adds courtesy and warmth to the moment.

Once the physician has presented the facts of the illness to the patient and shown that he is willing to answer questions, he need do no more than make brief visits once or twice a day. He should, however, make it clear to the family that loneliness adds to the anguish of dying. Since time immemorial, the deathwatch has been the custom in many nations. It is conducted by the patient's family and friends to insure around-the-clock coverage. Unfortunately, this custom has declined in recent years and probably the tempo of our times precludes its return. I have found that simply telling the family of this custom is all that is necessary to have them carry it out. One should also warn the relatives that the patient is apt to have bursts of anger, depression, and remorse. These should be handled just as they would be were he not dying. The family should also be appraised of the fluctuations in mood described before under the term middle knowledge.

Nausea and dyspnea in the dying are often difficult to treat. Oxygen, proper positioning, and a minimum of movement make breathing easier, but there is no good remedy. Nausea is sometimes helped by 50 cc of 95% ethyl alcohol given intravenously in 1000 cc dextrose and water by slow drip. The phenothiazines, particularly chlorpromazine (thorazine) and prochlorperazine (compazine), are potent antiemetics when given intramuscularly. There is widespread mention that marijuana or its active ingredient, tetrahydrocannabinol, is a most effective agent in controlling nausea. The use of transcendental meditation and hypnosis has added to the comfort of the dying. Both methods have safety as their principal recommendation. They are by no means universally beneficial, because only about 50% of the population can use such techniques to a therapeutic degree. Hypnosis in good subjects can be remarkably effective in combating anxiety, nausea, dyspnea, and pain.

Pain, although uncommon in terminal cancer, presents certain problems when present. Pain control should first be attempted through non-narcotic analgesics such as aspirin, acetaminophen (Tylenol®), and indomethacin (Indocin®) liberally potentiated by a benzodiazepine. Other palliative measures, e.g. heat, soaks, splints, and physiotherapy should be used as well. A large part of the effectiveness of pain therapy is in letting the patient know something is being done for him. Hypnosis and other relaxation techniques can be used. If the pain requires a narcotic, most physicians hesitate to prescribe one in a therapeutic dose range because they fear addiction. Unless their patient has a prior history of drug addiction, this fear is largely unwarranted. However, in order to allay the physician's doubts, there is a simple test to determine the patient's potential for addiction. Since the procedure for this test follows the protocol for treating pain, it requires no special measures.

If we accept the fact that pain can be heightened or reduced by a variety of psychological factors, the first step will be to shape the treatment in a way that will reduce the negative and enhance the positive therapeutic effect. This can be done by allowing the patient to obtain medication whenever he needs it. The nurse is instructed to comply with his wishes readily and never to question his need for pain relief. It should be taken orally if possible, but parenterally if not. The dose must be in the therapeutic range (75–100 mg meperidine, 15 mg morphine sulfate) and the order should allow three doses to be taken at half-hour intervals for a total of six per day. Under this regimen most patients end up taking less drug per day than if prescribed in the conventional way. After a week or more their intake will have reached a plateau. The potential addict will show a different intake course, one that slowly but steadily ascends without plateauing. The fact that the request for medication is not questioned and that they do not have to follow an arbitrary schedule set by a clock makes a difference. Pain tends to fluctuate according to the patient's level of distraction, depression, and interests. The need for medication clusters around specific points during the day with long hours of surcease in between. Knowing that the medication is there and will be given without contest reduces much of the grievous anticipation so commonly found in hospitals with rigid narcotic policies. Reducing dread reduces pain in turn. It has been my experience that once an individual becomes secure in the knowledge that medication is always ready when he needs it, his narcotic requirement drops.

Potentiating agents should be employed to prolong and increase the effectiveness of the narcotic. The phenothiazines have been used, as have the benzodiazepines and antihistamines. Less commonly used are the amphetamines. Five mg of dextroamphetamine sulfate once or twice a day can counteract the sedative effect of the narcotic, a property that is greatly appreciated by people who resent being made groggy.

Not infrequently, patients unwittingly employ a narcotic for sleep. This misuse can easily be detected by noticing an aggregation of medication requests around the hour of sleep. By ordering a tranquilizer in the hypnotic dose range, the bedtime narcotic can often be eliminated. We are fortunately blessed by an abundance of relatively safe medications for sleep. I favor the benzodiazepines such as flurazepam (Dalmane®) 30–45 mg/hs, diazepam (Valium®) 20–30 mg./hs, or oxazepam (Serax®) 30–45 mg/hs. Some individuals have used the tricyclic antidepressants. I have not been impressed by their use in this setting except as a hypnotic when given in a single bedtime dose.

It has always been my custom to speak with the nurses, dietitians, and attendants about the patient. So often, in the impersonal transactions of giving care—injecting, medicating, bathing, and changing dressings—the caretakers come to forget the person behind the patient. If there is something unique about the individual's life, something that will make him stand out to the staff, this should be relayed. There was, for example, an elderly man, quite shy and unappealing, whom the nurses treated well enough, but gave no extra attention. His chest and arms were emblazoned with curious tattoos. While talking with him, I learned he had been with the British army and had fought on the northwest frontier in Afghanistan. He was, in fact, one of the last remnants of the thin red line of British empire that once stretched around the world. Sharing this information with the nursing staff endowed him in their eyes with an aura of romance that belied his scrawny and miserable appearance. I think he received better care. Another woman, quite lackluster in personality and appearance, had consistently, over 40 years, won a prize for American Beauty roses in her city. Another gentleman had owned and bred appaloosa horses in the Southwest. These simple vignettes vivified the patient to the staff, reminded them that these dying shells had once been unique and special. It always surprises me how quickly staff interest picks up once they know something special about a patient.

In conclusion, the most important aspect of care in the treatment of the dying is to maintain open and honest communication between the doctor, the patient, and the family.

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# SOMATOSTATIN: PHYSIOLOGICAL AND CLINICAL SIGNIFICANCE

❖7207

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## INTRODUCTION

In 1972, while searching in hypothalamic extracts for the releasing factor for growth hormone (at that time and still uncharacterized[1] ), we observed that the material from some fractions of the purification sequence would powerfully inhibit the secretion of immunoreactive growth hormone. Krulich & McCann (6) had previously observed such an inhibitory activity, but no attempt at its characterization had been reported. Our test system was composed of sets of identical tissue-culture plates in which anterior pituitary cells from normal rats were attached as a monolayer four to six days after placing in the dish after dispersion with collagenase and trypsin (7).

The major component with the growth hormone release-inhibiting activity was isolated, characterized, reproduced by total synthesis, and named *somatostatin.* It has the following primary sequence (8–10):

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH.

Both the oxidized and the reduced forms of the peptide were shown to have the full biological activity in vitro or in vivo in various animal species (8, 10).

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[1] The literature and proceedings of several symposia on growth hormone secretion (1–3) contain reports claiming the isolation and characterization of growth hormone-releasing hormone (GHRH) as a decapeptide (4); biological activity was evidenced by a highly controversial assay based on pituitary "depletion" of bioassayable growth hormone. It was later shown (5) that GHRH was identical to a fragment from the N-terminal of the $\beta$ chain of hemoglobin. GHRH has no effect on the secretion of immunoreactive growth hormone, and is thus most likely an artifact.

## LABORATORY STUDIES

In laboratory animals, somatostatin inhibited growth hormone secretion acutely induced by all pharmacologic means, such as barbiturates, L-Dopa, arginine, and morphine (8, 11–13), as well as after electrical stimulation of the hypothalamus or amygdala (14) or catecholamine infusion in the third ventricle (12). Additionally, somatostatin was found to inhibit the secretion of thyrotropin induced by thyrotropin-releasing factor (TRF), while not affecting the concomitant secretion of prolactin triggered by the same dose of TRF (15, 16).

In some of the above studies, Koerker et al (12) noticed that infusion of somatostatin in nonanesthetized baboons was accompanied by hypoglycemia, which was associated with the prompt inhibition by somatostatin of the (basal) secretion of glucagon and insulin. This effect was clearly shown to be directly at the pancreatic level by in vitro infusion of the isolated pancreas (17–21), as well as with tissue cultures of the endocrine pancreas (22).

At both receptor sites (i.e. adenohypophysis and endocrine pancreas), somatostatin is significantly active at similar concentrations: $10^{-9}$ M in vitro, $\geq$ 200–400 ng/100 g body weight in vivo (23).

It had been observed in the earliest studies in vivo with somatostatin (8, 11) that the peptide had a very short biological half-life ($<$ 4 min). Also, with an exquisitely sensitive bioassay (16), and later by radioimmunoassay (24, 25), it was shown that somatostatin is present in parts of the central nervous system (CNS) other than the hypothalamus; indeed, the spinal cord appears to contain a high concentration. These observations led us to wonder whether the effects of somatostatin on the endocrine pancreas could, in physiological circumstances, truly be due to peptides of hypothalamic origin. A more satisfactory hypothesis would have been the possible release of somatostatin at (peptidergic) nerve endings in the neuroinsular complexes of the endocrine pancreas. Indeed, somatostatin activity was found in aqueous extracts of the fetal rat pancreas by bioassays (23) and later by radioimmunoassays (25). When this was investigated by histoimmunofluorescence, no nerve endings containing somatostatin were found; however, a discrete population of endocrine cells was found to contain somatostatin (26–29). These are different from the $\alpha$ cells secreting glucagon and the $\beta$ cells secreting insulin. Early evidence with the light microscope (26–28), and more recently with the electron microscope (29), have assigned the somatostatin-secreting cells of the pancreas to be the so-called D cells, for which no well-established function was available thus far. In the same reports (26–28), somatostatin-containing cells were observed (by immunofluorescence) in the duodenum, the fundic and antral mucosa of the stomach, and, although in much more reduced numbers, in the glandular elements of the jejunum and ileum and occasionally as isolated elements or small clusters in the exocrine pancreas. These observations have been confirmed in all species of vertebrates studied thus far (26).

Somatostatin has also been shown to inhibit the secretion of gastrin as induced by meals (30), and there are two reports that in relatively large doses, it can inhibit the secretion of secretin and of the exocrine pancreas as induced by HCl (31) and gastric secretion of HCl and pepsin (32), as induced by pentagastrin.

When administered to animals rendered diabetic by alloxan, streptozotocin, or pancreatectomy, somatostatin regularly produces dramatic lowering of hyperglycemia. This effect is always accompanied by inhibition of the basal elevated secretion of glucagon of either pancreatic or extrapancreatic origin (33). Somatostatin has no effect on the peripheral utilization or distribution of glucose (12, 34), nor is there evidence that it acts at the hepatic level to inhibit glycogenolysis or gluconeogenesis (35).

In early studies, somatostatin appeared to act distally to cyclic adenosine monophosphate (cAMP), since it inhibits release of growth hormone normally produced by both (dibutyryl)cAMP and theophylline (36, 37). It has been reported to lower cAMP levels and elevate cyclic guanosine monophosphate (cGMP) in pituitary tissues (37, 38). In acute studies, although somatostatin inhibits the release of preformed growth hormone, it does not inhibit the biosynthesis of growth hormone in the same pituitary cells (36).

With regard to earlier observations of somatostatin's presence in parts of the CNS other than the hypothalamus, there are recent reports of its effects on the spontaneous electrical activity of some neuronal systems as observed by microelectrode recordings (39) and on behavior (40, 41). It has been shown to counteract the seizure-inducing effects of strychnine (42) in the rat.

All of the above studies, as well as the clinical studies discussed below, have been conducted with somatostatin prepared by total synthesis (10). Series of synthetic analogues have already been prepared, including molecules with biological potency greater than that of the native substance (43). Evidence for dissociation of several of the multiple biological activities of some analogues of somatostatin at some receptor sites has recently been achieved (79, 80).

No evidence (macro- or microscopic) of toxicity, either acute or after 21-days' administration, has been seen in dogs, rats, mice, or Rhesus monkeys with well-characterized synthetic preparations of somatostatin administered in doses ten- to a hundredfold the human clinical doses on a body weight basis.[2] Baboons, in which somatostatin was chronically administered, were reported to have shown abnormal platelet function as well as various blood disorders with postmortem evidence of lung hepatization (44). A causal relationship, however, is not apparent. Substances other than somatostatin were also administered, and no strict controls are or were available to determine whether the animals, sitting for long periods of time in primate chairs and with multiple implanted catheters, may not have been prone to developing some type of coagulation disorder or latent septicemic infection responsible for death. This question is discussed further below.

## CLINICAL STUDIES IN MAN

### *Growth Hormone*

Somatostatin inhibits growth hormone responses to virtually all known physiological and pharmacological stimuli such as arginine (45–47), L-Dopa (45), insulin-induced hypoglycemia (47, 48), exercise (49), sleep (50), and meals (51, 52) in normal man (45–50) and in human subjects with growth hormone-producing pituitary tumors as in acromegaly (46–48, 51, 53–55). Although several studies (45–49, 56–58) have failed to demonstrate an effect on basal growth hormone levels in normal man, there is usually a rebound upon discontinuation of the somatostatin infusion, suggesting that inhibition of basal hormone secretion had been present (45, 46, 48, 49, 57). In pathological states associated with elevated basal growth hormone levels, such as acromegaly (46–48, 51, 53–55), protein-caloric malnutrition (55), and diabetes (49, 59), suppression is readily apparent.

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[2] All studies were carried out in compliance with FDA regulations in an application for an IND number, which was granted to one of us (RG) in April 1973.

The cyclic and linear forms of synthetic somatostatin appear to be equally effective inhibitors of growth hormone secretion. With intravenous administration, inhibition is demonstrable within 15 min (53, 54) and upon termination of the somatostatin infusion, growth hormone levels characteristically rebound above preinfusion levels. From the rapidity with which inhibition by somatostatin begins and its quick reversal after termination of the infusions, it is apparent that the action of somatostatin on growth hormone release is almost immediate and that it has a short biological half-life (4–8 min). When administered subcutaneously in doses up to 8 mg (57), the effect of somatostatin lasts for only 2 hr. Attempts to prolong its action by administering it subcutaneously in oil or gelatin (54) or after coupling it to protamine zinc (51) have not resulted in increases in activity beyond 4 hr. The time required for maximal suppression is a function of the fasting growth hormone level when conventional doses of somatostatin are used. In one study of acromegalic patients (54), doses between 100 and 1000 μg/hr were found to be maximally effective; a lesser response was observed with 25 μg/hr, and none with 10 μg/hr. No comparable data are available concerning the sensitivity of normal pituitary tissue.

Once suppressed, there has been no escape of growth hormone from inhibition during short-term studies and in longer studies carried out in acromegalic and diabetic subjects for as long as 28 hr (51, 59). It has been reported, however, that growth hormone responses to TRF and luteinizing hormone-releasing factor in acromegalic patients are not inhibited by somatostatin (60).

OTHER PITUITARY HORMONES In normal man, somatostatin does not affect the secretion of luteinizing hormone and follicle-stimulating hormone (45, 48, 53), adrenocorticotropin (48, 61), and prolactin (45, 48, 58). Thyrotropin responses to TRF are inhibited by somatostatin (48, 58, 62); most (45, 48, 53, 58), but not all (63), studies indicate that basal levels are apparently not affected.

Atypical responses to somatostatin may occur in patients with pituitary tumors. Thus, somatostatin has been reported to lower fasting prolactin levels in patients with acromegaly (53) and to lower ACTH levels in patients with Nelson's syndrome (61), although somatostatin does not affect secretion of these hormones by normal pituitary tissue.

### *Pancreatic Hormones*

Somatostatin inhibits secretion of both insulin (34, 46, 47, 53, 54, 56, 64–69) and glucagon (34, 46, 51, 52, 54, 56, 57, 59, 67–70) in man. Fasting insulin levels (34, 46, 47, 53, 65, 68), as well as insulin responses to meals (51), oral and intravenous glucose (46, 51, 54, 57, 66, 68), arginine (47, 56), glucagon, and tolbutamide (69), are diminished or completely prevented by somatostatin. Similarly, basal glucagon secretion (34, 46, 51, 52, 54, 59, 67, 68), as well as glucagon responses to meals (51, 52, 57), intravenous arginine (56), insulin-induced hypoglycemia (71), epinephrine (70), and insulin deprivation (59), are suppressed by somatostatin. The inhibitory effects of somatostatin on glucagon secretion occur with equal effectiveness in normal subjects (34, 56, 67, 68, 70), insulin-dependent diabetic subjects (52, 56, 57, 59), and patients with acromegaly (46, 51).

The ability of somatostatin to inhibit insulin and glucagon secretion has provided a useful tool for studying the physiological and pathological effects of these hormones (46, 52, 56, 57, 59, 67) on human metabolism (34). Infusion of somatostatin lowers plasma glucose levels in normal man despite concomitant lowering of both plasma insulin and glucagon levels (34, 46, 67). These observations provided the first clear-cut evidence that glucagon has an important physiological role in human carbohydrate homeostasis. Somatostatin itself has no direct effect on either hepatic glucose production (72) or peripheral glucose utilization (34, 73), since the fall in plasma glucose levels could be prevented by exogenous glucagon (69).

In juvenile-type diabetics, somatostatin diminishes fasting hyperglycemia by as much as 50% in the complete absence of circulating insulin (57, 74). Although somatostatin impairs carbohydrate tolerance after oral or intravenous glucose challenges in normal man by inhibiting insulin secretion (46, 51, 54, 64, 66), carbohydrate tolerance after ingestion of balanced meals is improved in patients with insulin-dependent diabetes mellitus through the suppression of excessive glucagon responses (52, 57). The combination of somatostatin and a suboptimal amount of exogenous insulin (which by itself had prevented neither excessive hyperglycemia nor hyperglucagonemia in response to meals) completely prevents plasma glucose levels from rising after meal ingestion in insulin-dependent diabetics (52). Through its suppression of glucagon and growth hormone secretion, somatostatin has also been shown to moderate or prevent completely the development of diabetic ketoacidosis after the acute withdrawal of insulin from patients with insulin-dependent diabetes mellitus (59).

Pancreatic tumors secreting either glucagon (54) or insulin (75) have been reported to be responsive to somatostatin. Curiously, in patients with insulinomas, basal and glucagon-stimulated insulin secretion is inhibited by somatostatin, but that due to tolbutamide characteristically is not (75). Infusion of somatostatin in such patients must be performed cautiously since, despite lowering of basal insulin levels, plasma glucose levels fall precipitously.

### *Gastrointestinal Hormones*

Data concerning the effect of somatostatin on gastrointestinal hormones in man are presently available only for gastrin (30). Somatostatin suppressed fasting plasma gastrin levels approximately 40% in normal subjects and in subjects with acromegaly during a 60-min infusion. Additionally, gastrin responses after ingestion of a balanced meal were completely suppressed. The markedly elevated fasting gastrin levels in two patients with pernicious anemia were diminished by more than 50%

during a 75-min infusion of somatostatin. Finally, both gastric acid secretion and extreme hypergastrinemia of a patient with Zollinger-Ellison syndrome were suppressed during a 20-min infusion of somatostatin (30).

### *Side Effects and Toxicity*

Although some adverse hematological effects have been reported in baboons (44) and rabbits (76) receiving large amounts of somatostatin, no serious toxicity has been encountered in man during a two-year clinical experience in over 300 subjects. The most frequent side effects encountered are transient nausea, occasional diarrhea, and abdominal cramps (50, 56, 58). Transient falls in blood pressure (10 mm Hg for 10 min) and rises in pulse (10 beats per min lasting 10 min) are sometimes observed (49, 56). No significant hypoglycemia has been found, except in diabetics given exogenous insulin. No changes in white blood cell count (46, 48), platelet count (46, 77), bleeding time (77), prothrombin time (77), partial thromboplastin time (77), serum electrolyte levels (46, 48), or renal or hepatic function (46, 48) have been encountered either in acute studies or after prolonged (12 hr) infusions of somatostatin. All these values have been found to be normal after intermittent administration of somatostatin as frequently as once weekly for over a year (77). Although a recent report (78) suggested that somatostatin might diminish platelet aggregation, similar studies using higher doses of somatostatin found no effect (77); these discrepant results might have been due to the use of different preparations of somatostatin. Since bleeding times were unaffected in both studies, the clinical significance of the reported platelet abnormalities is unclear.

### *Therapeutic Implications*

From the foregoing description of the ability of somatostatin to inhibit the secretion of various hormones, it would appear that this agent may be of therapeutic use in certain clinical conditions such as acromegaly, pancreatic islet cell tumors, and diabetes mellitus. With regard to endocrine tumors, it must be emphasized that while somatostatin will inhibit hormone secretion by these tissues, it would not be expected to diminish tumor growth (36, 81). Thus, in these conditions it is unlikely that somatostatin will find use other than as a symptomatic or temporizing measure.

In diabetes mellitus, however, somatostatin might be of considerable clinical value. First, it has already been demonstrated that it can acutely improve fasting (57) as well as postprandial hyperglycemia (52, 57) in insulin-requiring diabetics by inhibiting glucagon secretion. Second, since growth hormone has been implicated in the development of diabetic retinopathy, the inhibition of growth hormone secretion by somatostatin may lessen this complication of diabetes. Finally, through suppression of both growth hormone and glucagon secretion, somatostatin may prevent or diminish the severity of diabetic ketoacidosis and find application in "brittle diabetes." These optimistic expectations must be tempered with the facts that the diverse effects of somatostatin on hormone secretion and its short duration of action make its clinical use impractical at the present time and that its long-term effectiveness and safety have not been established as yet.

## CONCLUSION

Somatostatin, originally characterized on the basis of one well-defined biological activity and extracted from one well-defined area of the brain, has now been shown to exist in significant quantities outside the CNS and to be polyvalent in its ability to inhibit hormone secretion. Its tissue distribution is not random: the peptide is found both in the CNS and in highly specialized (neuro-) endocrine cells ontogenically derived from neural crest primordia. The multiple effects of the peptide are all consistent, as they appear to involve inhibition of the secretion of polypeptides; in all cases, the target or receptor is topographically closely related to the secretory element. Somatostatin may well represent the prototype of a new class of carriers of physiological information (for which one of us is proposing elsewhere the generic name of *cybernins*) different from classical hormones (e.g. ACTH, gonadotropins, insulin, etc) and different also from the classical neurotransmitters (e.g. catecholamines, dopamine, serotonin, and acetylcholine). The existence of such a new class of agents seems likely, but remains to be firmly established; methodology for this is just becoming available as this review is being prepared. Abnormalities in the metabolism or secretion of these agents could be significant in the etiology of malfunction or disease of various organic systems. For example, if somatostatin is actually synthesized in multiple areas of the CNS, as seems to be the case, local dysfunctions in the biosynthesis or release mechanism for somatostatin could be involved in the pathogenesis of some mental disorders.

The possible clinical significance of somatostatin, particularly as a component of the treatment of diabetes, also remains to be established in further clinical studies. The multiplicity of target receptors for the peptide and its short duration of action will require careful assessment of dosage and timing of administration and knowledge of differential affinities of the various receptors for the synthetic replicates of the native primary sequence or some of its synthetic analogues.

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# NEUROENDOCRINE DYSFUNCTION IN DEPRESSIVE ILLNESS

❖7208

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## INTRODUCTION

There are at least two reasons for suspecting that neuroendocrine regulation might be disturbed in patients suffering severe depressive illness. First, the clinical syndrome is characterized by symptoms suggesting hypothalamic dysfunction—disturbances in mood, appetite, sexual and aggressive drives, autonomic activity, sleep, and frequently, a diurnal variation in symptomatology; it would be plausible to hypothesize that hypothalamic regulation of neuroendocrine function might also be affected. Second, the neurotransmitters currently implicated in the chemical pathology of depressive states (1)—noradrenalin, serotonin, and dopamine—also play a major role in regulating the activity of hypothalamic neuroendocrine cells controlling secretion of pituitary hormones (2).

In this brief review, we focus on evidence that the regulation of adrenocorticotropic hormone (ACTH), human growth hormone (HGH), luteinizing hormone (LH), and thyroid stimulating hormone (TSH) responses are abnormal in certain groups of depressed patients. Such data are important for the internist to take into account in the medical evaluation of the depressed patient whose somatic complaints may suggest an underlying metabolic or endocrine abnormality.

## CORTISOL

Cortisol (with its derivatives) has been the most extensively studied hormone in patients suffering clinical depressions; indeed, the first papers appeared more than two decades ago. Refinements over the years in biochemical assays, endocrine function tests, psychiatric classification systems, experimental designs, and control procedures have resulted in considerable clarification of diverse findings that were once apparently contradictory. To summarize the numerous reports, which are reviewed in detail elsewhere (3–5):

1. A large minority of depressed patients hypersecrete cortisol.
2. Factors of psychological distress and emotional arousal account for some of the excessive cortisol secretion, especially in patients suffering neurotic, reactive depressions.
3. Many depressed patients, however, manifest a pervasive substantial hypersecretion of cortisol, apparently unrelated to measures of psychological stress, suggesting an underlying neuroendocrine abnormality. Such patients are most commonly those with severe illnesses of the "endogenomorphic" (6) and psychotic types—that is, with syndromes characterized by a pervasive loss of interest and pleasure, depressed mood, reduced emotional responsivity to the environment, psychomotor retardation or agitation, pessimism, "hypothalamic" signs, and frequently, guilty and suicidal preoccupations that may reach delusional proportions.
4. The cortisol hypersecretion ceases after clinical recovery.

The most recent studies in this area have focused on abnormalities in the 24-hr plasma cortisol pattern and its responsiveness to dexamethasone suppression tests.

In the normal subject (and in the depressed patient after recovery) cortisol is secreted daily in a series of about seven to nine discrete episodes, reflected in spikes in plasma cortisol concentration and synchronized with the sleep-wake cycle (7). In a subject sleeping from 12 midnight to 8 AM, the first episode occurs about 2 AM, the largest episodes occur between 5 and 9 AM, and cortisol secretion virtually ceases for about six hours after 8 PM. By about midnight, plasma cortisol concentration approaches zero. In certain severely depressed patients, this secretory pattern is altered: the number of episodes is generally increased, and plasma cortisol concentration is elevated both at the beginning and end of secretory episodes (Figure 1; 8). In our study, in which plasma cortisol was determined every 20 min for 24 hr, mean 24-hr plasma cortisol concentration was 11.25 μg/100 ml [standard deviation (SD) 1.83] in 7 severely depressed patients, compared with 7.28 μg/100 ml (SD 1.87) for 54 normal subjects (9). An especially noteworthy observation was that cortisol secretion continued actively during the late evening and early morning hours when secretion is normally minimal. A flattening of the circadian plasma cortisol curve results, with the late evening and early morning segment appearing especially abnormal (Figure 2).

It appears doubtful that this distortion of the secretory pattern could be attributed to a simple stress response, since the hypersecretion persists during periods of sleep (9), occurs sometimes in patients with little evidence of affective arousal (9), is not corrected by sedative medication (10), and is most abnormal in the late evening, rather than in the morning when depressive symptomatology is frequently most intense.

Since the abnormalities in cortisol secretion are at times severe enough to resemble those seen in Cushing's syndrome, recent studies of responses to dexamethasone suppression tests in depressed patients are especially interesting (5). After a late evening oral dose of 2 mg of dexamethasone, plasma cortisol concentration is normally suppressed below 6 μg/100 ml for 48 hr. In the hypersecreting depressed

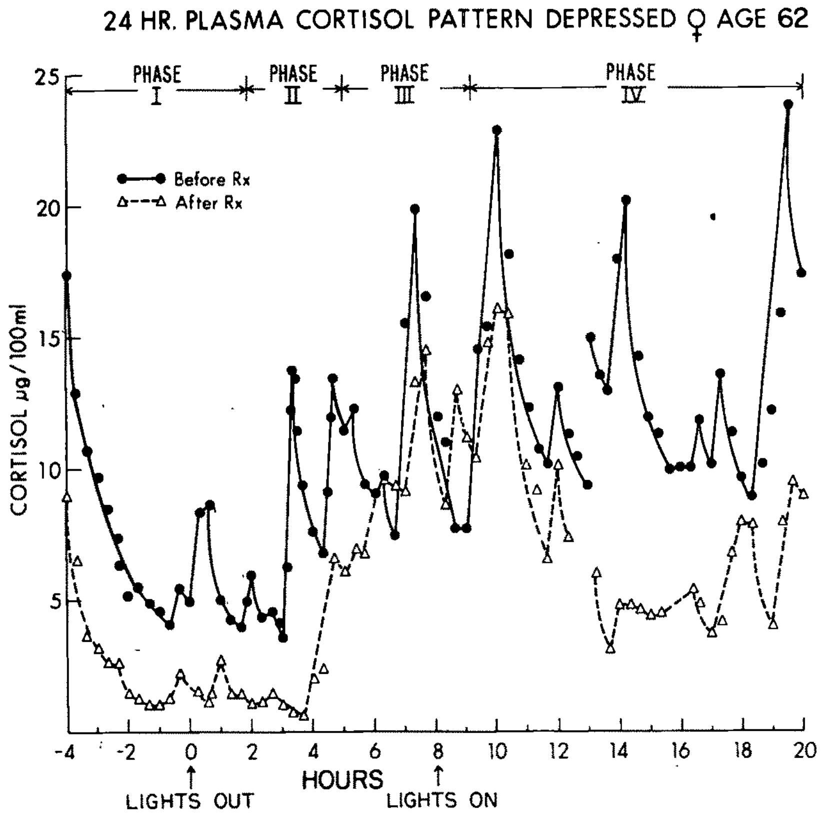

*Figure 1* Plasma cortisol concentrations determined every 20 min for 24 hr in a severely depressed 62-year-old woman before and after clinical recovery. Zero time is the hour of lights out for sleep. After clinical recovery, the plasma cortisol pattern is entirely normal and is characterized by a cessation of cortisol secretion for several hours before and after sleep onset. During depression, hypersecretion and disruption of the pattern are evident.

patient, however, plasma cortisol concentration is resistant to dexamethasone suppression, particularly in the late evening of the day after dexamethasone ingestion. As clinical recovery proceeds, normal responsivity of cortisol to dexamethasone is restored.

Although the cortisol hypersecretion and relative resistance to dexamethasone suppression in depressive illness sometimes approaches Cushingoid proportions, it is interesting, and puzzling, that depressed patients never show the physical stigmata of Cushing's syndrome.

In all likelihood, the cortisol hypersecretion reflects ACTH hypersecretion, which in turn reflects hyperactivity of the neuroendocrine cells secreting corticotropin releasing hormone (CRH). Efforts to link this neuroendocrine abnormality to cur-

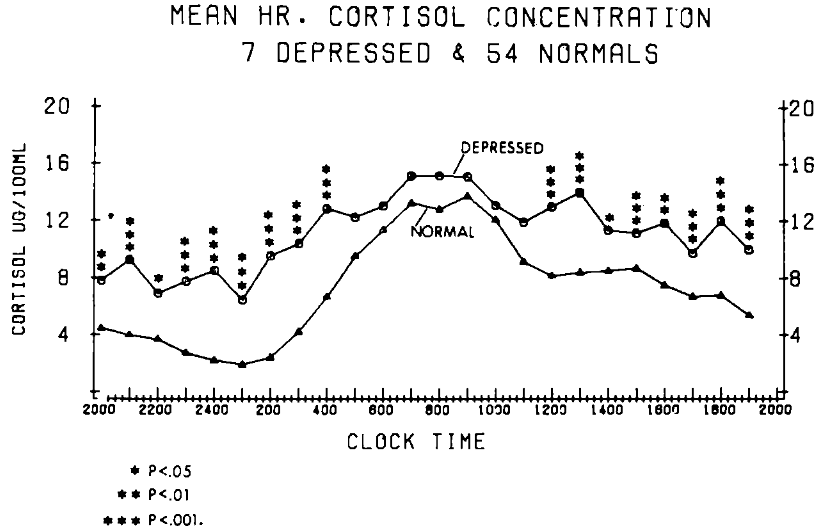

*Figure 2* Mean hourly cortisol concentration in 7 severely depressed patients and 54 normals (the latter data provided by E. D. Weitzman). The largest and most significant differences between the patients and normals are in the afternoon, evening, and early morning hours

rent theories of brain neurotransmitter disturbances in depressive illness are, of course, entirely speculative at this time, because of uncertainty both about the pathoneurochemistry of depression, and about the precise neurotransmitter regulation of CRH. However, since one of the major neurochemical hypotheses of depression suggests a functional depletion of brain noradrenalin (1), it is important to note that there is considerable neuropharmacological evidence for a brain noradrenergic system that exerts a tonic inhibition of ACTH secretion (11). It is tempting to speculate that the cortisol hypersecretion in depressed patients reflects a disinhibition of CRH secretion secondary to hypothalamic noradrenalin depletion.

## HUMAN GROWTH HORMONE (HGH)

Growth hormone is physiologically secreted in association with slow-wave sleep, falling blood sugar, exercise, and anxiety; in addition, it is stimulated by infusions of arginine and by a variety of pharmacological agonists of dopamine, noradrenalin, and serotonin (e.g. apomorphine, L-dopa, clonidine, and 5-hydroxytryptophan (2, 12, 13).

Most recent research on HGH secretion in depression has focused on responses to L-dopa ingestion and to insulin-induced hypoglycemia.

In addition to the usual manifold problems of biologic study in groups of psychiatric patients, analysis of HGH responses are complicated further by the need to control for variables of diet, obesity, age, sex, and estrogen potentiation. Thus, the apparently diminished HGH responses to L-dopa in depressed patients proved to be related not to their depression but to the high proportion of postmenopausal women among them; HGH responses are reduced after menopause, almost certainly because of diminished estrogen potentiation (14). On the other hand, HGH responses to a standard insulin-induced hypoglycemia provocation test have been found to be significantly reduced in patients suffering depressive illness (15–17), and a recent study demonstrated that postmenopausal women suffering primary unipolar depressive illnesses had substantially diminished HGH responses compared with an age-matched control group of normal postmenopausal women (18). The minimum clinically adequate response is usually considered to be greater than 5 ng/ml after at least a 50% drop in blood glucose; a substantial proportion of depressed patients fail to achieve this level.

The HGH response to hypoglycemia is probably mediated in large measure by brain catecholamines, although serotonin may also play a role (2, 12). The deficient HGH responses in depression may also, then, be related to the hypothesized biogenic amine disturbance. While the hypersecretion of cortisol normalizes after clinical recovery, it appears that the deficient HGH responses to hypoglycemia persist into remission in some patients and may conceivably be related to the biologic vulnerability to depressive illness.

## LUTEINIZING HORMONE (LH)

Very little has been reported as yet about LH secretion in depressed patients. In one recent study, plasma LH concentration in postmenopausal women with primary unipolar depressive illness was significantly less (30% lower) than in age-matched normal postmenopausal women (19). Preliminary evidence suggests that this relative LH reduction persists into remission. Since noradrenalin regulates the rise in LH after gonadectomy (20) (and presumably after menopause—a functional gonadectomy), noradrenalin depletion might be implicated in the reduced LH levels in postmenopausal depression.

## THYROID HORMONE

All clinical and biochemical measures of thyroid secretion have been found to be normal in depressed patients, including ankle reflex time and plasma $T_4$, $T_3$, and TSH concentration (21). On the other hand, several laboratories have reported that the TSH response to an infusion of a standard dose of thyrotropin releasing hormone (TRH) is significantly reduced in depressed patients compared to normal control subjects (22–24). Since the TSH response to TRH is almost surely a purely pituitary function, the relation of this observation to hypothalamic function in depression remains obscure.

It is also well established that small daily doses of $T_3$ (25 $\mu$g) significantly potentiate the therapeutic efficacy of tricyclic antidepressant drugs in depressed women (25, 26). Again, the mechanism of this potentiation is unknown, although one hypothesis suggests that $T_3$ sensitizes brain receptors to noradrenalin, facilitating the noradrenergic action of the antidepressant drugs.

## OTHER HORMONES

There have been very few published studies of other hormones in depressed patients, and those that have appeared have generally reported no significant abnormalities. Plasma prolactin concentration was found to be slightly elevated in depressed patients compared to normal subjects, but this was felt to represent a mild stress response, and prolactin suppression after L-dopa ingestion was normal in these cases (27). A recent study reported that the prolactin response to a standard infusion of TRH was augmented in depressed patients (24). Once again, the relation of this deviant pituitary response to possible hypothalamic dysfunction in depression is unknown.

Plasma and urinary testosterone levels were found to be in the normal range in depressed men, and did not change after clinical recovery (28).

Plasma estrogens and progesterone have been studied somewhat in relation to premenstrual depressive moods, but there is relatively little information as yet that would suggest any abnormality in depressive illness per se.

## SUMMARY

Patients with severe depressive illnesses of the endogenomorphic type frequently manifest several hormonal abnormalities. The best documented are the hypersecretion of cortisol, particularly in the late evening and early morning, associated with relative resistance to dexamethasone suppression; diminished or absent HGH responses to insulin-induced hypoglycemia; and diminished TSH responses to TRH. There are also reports from one laboratory of relatively diminished plasma LH concentration in postmenopausal women with primary unipolar depressive illness, a finding that awaits replication by other centers.

Most of these hormonal abnormalities provide further support to the concept of hypothalamic dysfunction in certain forms of depressive illness, and are consistent with current hypotheses of abnormal brain monoamine activity in these conditions. These studies suggest that analysis of neuroendocrine responses in patients with mental illnesses may be a valuable tool for the study of possible alterations in brain function. Further advances may be expected when radioimmunoassay techniques become available for the hypothalamic releasing and inhibiting factors themselves.

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# METABOLIC COMPLICATIONS OF JEJUNOILEAL BYPASS OPERATIONS FOR MORBID OBESITY

❖7209

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## INTRODUCTION

The basis for a surgical approach to massive obesity lies in the dual premise that severe obesity is a serious life-shortening disorder and that long-term medical therapy is usually unsatisfactory. Although various operative procedures have been suggested, reduction of the absorptive surface of the small bowel by extensive jejunoileal bypass has been used most widely. During the past nine years we have made a clinical and metabolic investigation of morbid obesity and the role of surgical management; over 150 obese patients have been treated by extensive jejunoileal bypass. This brief review of the problems and response to operation summarizes the metabolic complications that occurred.

The patients were selected for operation from a much larger group of obese subjects after an in-hospital appraisal of clinical, endocrinologic, metabolic, psychiatric, and body compositional status according to the following criteria:

1. Obesity of massive degree (weights of two to three times ideal levels) of at least five years duration.
2. Validation from attending physician of failure of dietary efforts to correct obesity over a period of years.
3. Evidence in the patient's history and evaluation of incapability to adhere to prescribed dietary regimens and/or exercise programs.
4. Absence of any correctable endocrinopathy such as hypothyroidism or Cushing's syndrome.
5. Absence of any other unrelated significant disease that might increase operative risk.

6. Presence of certain complications such as Pickwickian syndrome, hyperlipidemia, adult onset of diabetes, and hypertension that might be alleviated by significant weight reduction.
7. Assurance of patient's cooperation in conduct of pre- and postoperative nutritional studies and prolonged follow-up evaluation.

In the 150 massively obese patients who were selected by the above criteria and submitted to jejunoileal bypass, ages ranged from 16 to 63 (average was 36 years). There were 87 women and 63 men. Maximal weights prior to operation ranged from 280 to 600 pounds (127 to 272 kg). Immediately before operation weights ranged from 240 to 560 pounds (109 to 255 kg). Each patient was admitted to the hospital prior to operation for baseline study to detect possible endocrine causes of obesity, evaluate cardiopulmonary function, assess absorptive and nutritional status, appraise psychiatric and psychologic profile, and to make isotopic measurements of body composition.

A family history of obesity was elicited in about half of the patients. About one half had hyperlipidemia, exhibited by abnormal lipoprotein electrophoretograms in 60 patients with Fredrickson's Type IV hyperlipoproteinemia, 12 patients with Type II, and 1 with Type V. Gallstones occurred in 32%, maturity onset diabetes in 28%, persistent diastolic hypertension in 15%, and clinically active gout requiring treatment in 5%. Excluding diabetes, no significant endocrinopathies were uncovered. Body compositional data based on $^{40}K$ whole-body counting indicated an average body fat content in the range of 60–65% of body weight. Most patients had parameters of pulmonary function in the normal range except for a diminished $PaO_2$; six had clinical features of Pickwickian syndrome. Two patients had had previous myocardial infarctions and one had sustained a previous stroke.

The end-to-side jejunoileal shunt described by Payne (1) (Group 1) was used in the first 11 patients of this series but abandoned after disappointing follow-up experience. Subsequently, we have used end-to-end anastomosis of a short segment of proximal jejunum to a short segment of distal ileum, while draining the bypassed long length of jejunoileum into transverse colon or sigmoid. (2) In an effort to determine optimal dimensions of shortened bowel in alimentary continuity, three variations of this procedure have been studied: In 12 patients (Group 2) 12 inches of proximal jejunum were anastomosed end-to-end to the distal 12 inches of ileum (30 cm to 30 cm). In Group 3 in 21 patients the proximal 12 inches of jejunum were joined to ileum 6 inches proximal to the ileocecal valve (30 cm to 15 cm). In Group 4 in over 100 patients the anastomosis of proximal jejunum 12 inches from Treitz's ligament was made to ileum 8 inches from the ileocecal valve (30 cm to 20 cm).

In the majority of patients, recovery from operation was uneventful. However, there were five postoperative deaths in the series of 150 patients: one with bowel infarction from mesenteric venous thrombosis after intestinal bypass and extensive ileal and mesenteric resection for malignant ileal carcinoid with nodal metastases to the root of the mesentery; three from pulmonary embolism; and a fifth from diffuse thrombophlebitis, hemorrhagic pneumonia, and sepsis. The operative mortality rate was thus 3%.

Nonfat postoperative complications developed in 31 patients. They included wound seroma in 13 patients and superficial wound infection in 14 others. One of these also developed stomal obstruction, which required revision, and venous thrombosis with pulmonary emboli. One other patient had thromboembolic complications. Two patients developed early postoperative hepatitis due, we believe, to halothane anesthesia. There was only one early (first day) evisceration. All patients with these complications recovered with appropriate treatment.

Nasogastric suction was discontinued two to three days after operation and oral liquids, usually restricted to 1500 ml daily, were started. In the first few days after institution of oral liquids, multiple watery stools occured. Low fat diet, restriction of fluids with meals, and diphenoxylate hydrochloride in a dosage of 5 mg three to four times a day or codeine 15–30 mg three to four times a day usually resulted in satisfactory control of the diarrhea during the early postoperative period.

After discharge from the hospital each patient has been followed in conjunction with the family physician and in our clinic. An effort has been made to have all patients return repeatedly for both outpatient and inpatient studies of their clinical result; nutritional, metabolic, and body compositional status; psychiatric state; and degree of rehabilitation. Details of these results have been reported separately and exceed the scope of this report (3–6).

## RESULTS

Periods of follow-up in the groups under study range from five to eight years in the patients with Payne's operation (Group 1), from three to four years in the patients of Group 2, from two to three years in the patients of Group 3 (30 cm to 15 cm), and up to two years in the patients with the 30 cm to 20 cm jejunoileal shunt of Group 4. The data comparing weight loss after jejunoileal bypass in the patients of these several groups are presented in Figure 1. Although early precipitate weight loss occurred in each group, those with Payne's operation (Group 1) and 50% of those with 30 cm to 30 cm end-to-end jejunoileal shunt (Group 2) subsequently lost weight more gradually and weights tended to plateau after 12 to 18 months at levels above ideal range. After two or more years a few patients in both of these groups had begun to regain weight. In contrast, patients in the groups of end-to-end jejunoileal shunt with 30 cm of jejunum joined to 15 or 20 cm of distal ileum (Groups 3 and 4) have had a more rapid rate of weight loss during the first year after operation. More than 70% of patients in these two groups followed for one year or more have achieved reductions to the range of ideal weight.

Studies of body composition after jejunoileal bypass indicate that both body fat and lean body mass are lost in the early postoperative months. The loss of lean body mass has been greatest in Group 3 (30 cm to 15 cm), concomitant with the most prolonged persistence of diarrhea. In all groups, however, potassium homeostasis has been usually achieved by four to six months and subsequently fat is lost in preference to lean body mass (4).

During the follow-up period of a few months to eight years there have been 5 deaths among the 145 patients who have survived jejunoileal shunt. The results of

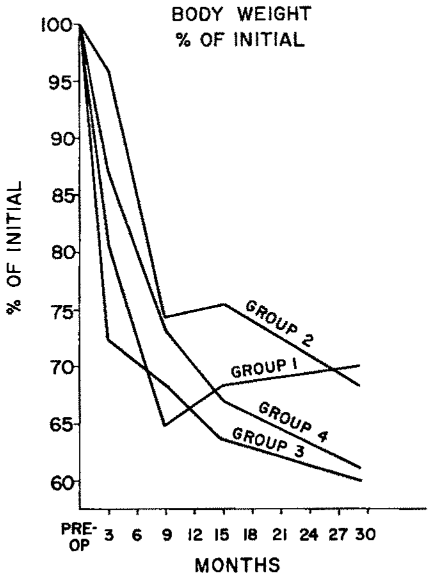

*Figure 1* Body weight plotted as percentage of initial weight against time in the four groups of this study.

clinical follow-up based on the criteria listed in Table 1 have been appraised in the surviving patients in each group followed for one year or more after jejunoileal bypass and are summarized in Table 2. In 11 patients with Payne's end-to-side jejunoileal shunt (Group 1), followed five to eight years, the absence of good results and the predominance of poor results reflect the failure of satisfactory sustained weight reduction. In the 92 patients with end-to-end jejunoileal shunt who have been followed from one to four years the results have varied with the dimensions of surgical shortening of intestine. In Group 2 (30 cm to 30 cm), 6 of the 12 patients (50%) achieved a good result. Fair results, achieved by 5 of this group, were due to failure to attain satisfactory weight reduction to the ideal range. No significant

Table 1 Criteria in appraisal of results

| | |
|---|---|
| Good | Weight loss satisfactory<br>No diarrhea problem<br>No metabolic deficits |
| Fair | Weight loss not ideal<br>Mild diarrhea on occasion<br>Minimal metabolic deficits |
| Poor | Weight loss unsatisfactory<br>Persistent diarrhea<br>Severe metabolic deficits |

Table 2 Jejunoileal bypass for morbid obesity (1966–1975): Results of follow-up

| Jejunoileal shunt technique | Number of patients | Years | Results Good | Results Fair | Results Poor |
|---|---|---|---|---|---|
| End-to-side | | | | | |
| (Payne procedure) 35 cm to 10 cm (Group 1) | 11 | 5–8 | 0 | 2 | 9 |
| End-to-end | | | | | |
| 30 cm to 30 cm (Group 2) | 12 | 3–4 | 6 | 5 | 1 |
| 30 cm to 15 cm (Group 3) | 20 | 2–3 | 12 | 7 | 1 |
| 30 cm to 20 cm (Group 4) | 60 | 1–2 | 49 | 8 | 3 |

metabolic or nutritional deficits have developed in any of these patients and diarrhea has not persisted as a problem. One patient had a poor result because of totally inadequate weight loss.

Among the 80 patients in Groups 3 (30 cm to 15 cm) and 4 (30 cm to 20 cm) who have been followed one to three years, 61 (75%) have achieved good results. Good results have been obtained by 81% of the 60 patients in Group 4 who have had follow-ups of one to two years.

The degree of rehabilitation in the patients who have had good results and most of those who are credited with fair results is difficult to measure objectively. Return to full preoperative work capacity and the assumption of full- or part-time new work has been accomplished by all but six patients. In the latter small group, retirement programs or wealth that precluded the need to work have been important influences. The new body image developed by those individuals who have brought their massively overweight status down to a normal or near normal weight range has been impressive to our medical and surgical team and in particular to our clinical psychologists and psychiatric consultants. A study of personality profiles and psychologic aspects of the massively obese patients of this group before and after jejunoileal bypass will be reported separately (5, 6).

Metabolic parameters that were assessed prior to operation have been studied in the period of follow-up. Reduction in carbohydrate absorption is indicated by impairment in D-xylose absorption and by flattening of oral glucose tolerance curves in the postoperative period. A large increase in fecal fat loss (20–85 g/day) has occurred in each of the 30 patients in whom it has been possible to measure this in the follow-up period after jejunoileal bypass (3). Malabsorption of fat and bile acids has been accompanied by an impressive and sustained fall in the serum cholesterol and triglyceride levels in all groups of patients. Irrespective of baseline concentrations there has been a consistent fall in both serum cholesterol and serum triglyceride to levels of 160 mg/100 ml or less after operation and these levels have been sustained throughout the periods of follow-up. Lipoprotein electrophoretograms have returned to normal in patients with Type II hyperlipoproteinemia and in all but four patients with Type IV. Sequential follow-up has shown no recurrence of the original lipoprotein abnormality in any patient (7).

### *Deaths During Follow-up*

An enumeration of the 5 deaths that have occurred in the 145 survivors of jejunoileal bypass follows. One young woman who made an apparently uneventful recovery from operation died suddenly in her home three months later. No autopsy was done. A man of 60 years with severe generalized atherosclerosis died with myocardial infarction in his home six months after operation. A young man of 25 years was victimized by alcohol abuse and died with variceal hemorrhage, alcoholic hepatosis, and liver failure seven months after operation. Two other patients, both women, ages 57 years and 34 years, died 15 months and 6 months, respectively, after operation with unexplained hepatitis and hepatic failure. In the older woman there was a history of "social" drinking but no evidence of alcohol abuse. The other woman was a total abstainer. Overall mortality rate in 150 patients was 6.6%.

### *Persistent Diarrhea: Water and Electrolyte Deficits*

Diarrhea ceased within one to three months after bypass in all patients in Group 2 (30 cm to 30 cm) and in eight of 11 patients in Group 1 excepting overindulgence in excessive liquid or fat intake. Postoperative diarrhea has persisted for a slightly longer period in most of the patients of Group 3 and 4. Persistent diarrhea has been a problem for 45% of patients in Group 3 (30 cm to 15 cm). In contrast, only 17% of the patients in Group 4 (30 cm to 20 cm) have had this problem. Electrolyte deficits requiring supplementation have only occurred in patients with persistent diarrhea. The major cations, potassium, magnesium, and calcium, have been given as oral supplements as required in such cases until the diarrhea has come under control; however, seven patients with more severe deficits have required hospitalization during the period of follow-up for parenteral supplementation.

Excessive intake of liquids with meals and of foods rich in fat and lactose have been frequent factors in refractory diarrhea. After 12 to 18 months, the postoperative adaptive changes in the shortened bowel described by Wright & Tilson (8) that increase absorptive capability not only curtail continued weight loss but increase dietary fat and lactose tolerance, and diarrhea of earlier months usually ceases. Buchwald et al (9) advise routine oral supplementation after jejunoileal bypass with 45–60 meq of potassium and 6 to 12 g of calcium carbonate daily in prophylactic therapy.

### *Fatty Liver and Hypoproteinemia*

Accumulation of neutral fat in hepatocytes is a common finding in massive obesity. In 100 obese patients submitted to wedge biopsy of the liver, we found gross and microscopic evidence of hepatic steatosis in 74% prior to jejunoileal bypass. Similar observations have been made by many authors, but the causative mechanism remains obscure (1, 9–11). After jejunoileal bypass an increase in fat accumulation in the liver in most patients has been documented by many observers (1, 9–11) Serum protein concentrations have tended to decline with notably lowered albumin levels (3). This appears to reflect protein-calorie malabsorption. The enhanced fatty metamorphosis of the liver coupled with protein-calorie malnutrition resembles an adult form of the tropical protein deficiency disorder known as kwashiorkor (12). Our clinical and body compositional data (4) support the concept that massively obese patients treated by jejunoileal bypass will recover satisfactorily from this phase of protein deficiency and hepatic fatty metamorphosis after 12–24 months if no added hepatotoxic factors are imposed.

### *Hepatotoxic Factors and Hepatic Failure*

Excessive alcohol intake after jejunoileal bypass led to alcoholic hepatosis, variceal hemorrhage, and death from hepatic failure in one patient and alcohol probably contributed to death from hepatic failure in one other patient. Alcohol abuse promoted hepatomegaly in three other patients in Group 1, with relief by stopping drinking in two and by restoring alimentary continuity in one other. We advise all patients to abstain from alcohol after jejunoileal bypass.

Another possible cause of hepatic injury after jejunoileal bypass is the potential for bacterial colonization and overgrowth in the shortened bowel and/or bypassed jejunoileum with toxic absorption of bacterial metabolites, deconjugates such as lithocolic acid, or bacterial endotoxin. The experimental work of O'Leary and his associates (13, 14) supports this possibility and suggests the use of broad spectrum antibiotics in preventive therapy. Careful close clinical observation of all patients who are submitted to jejunoileal bypass is mandated by the rare but hazardous potential for development of toxic damage to the liver, especially during the phase of protein-calorie malnutrition. The clinical symptoms are those of a "flu-like" syndrome of malaise, myalgias, weakness, and easy fatigue coupled with anorexia and nausea. Elevation of serum alkaline phosphatases, lactic dehydrogenases, and transaminases (SGOT) usually precedes a rise in bilirubin or clinical jaundice. Australia antigen is usually negative. In six patients in the present series who developed these clinical manifestations within 3 to 12 months after jejunoileal bypass, treatment with 3–8 weeks of tetracyclines (or doxycycline) coupled with elemental dietary supplements has resulted in subsidence of symptoms and reversion of the serum enzyme alterations and liver biopsies to normal. Failure of prompt response to such treatment is an indication to take down the intestinal bypass and restore alimentary continuity. This has been necessary in one patient in this series with subsequent subsidence of symptoms and early beneficial results. Failure to take down the shunt in two other patients alluded to previously whose response to tetracyclines and elemental diet was inadequate by retrospective analysis resulted in death from hepatic failure in each instance. Similar experience has been reported by Woodward's group (13, 14, E. R. Woodward, personal communication.)

In the early years of this series halothane anesthesia was used commonly. Two young women developed Australia antigen negative hepatitis in the immediate postoperative period following jejunoileal bypass under halothane. Each recovered fully without evidence of liver damage with supportive therapy. No hepatotoxic agent could be elicited to explain the hepatitis other than halothane. We have discontinued the use of this agent as an anesthetic in obese subjects.

### *Gallstones*

Gallstones occurred before jejunoileal bypass in 48 of 150 patients (32%); cholecystectomy was done in each. In the period of followup after bypass five other patients have developed symptomatic cholelithiasis and have had cholecystectomy. Neshat & Flye (15) have recently described a patient thought to have developed gallstones five weeks after jejunoileal bypass. Our experience suggests that it is more likely in such a case that stones were already present before operation. Whether bile becomes more or less lithogenic after jejunoileal bypass is a topic for investigation.

### *Renal Stones and Hyperoxaluria*

One or more bouts of ureteral colic attributed to stone formation have occurred in 12 of 150 patients after jejunoileal bypass. Hyperoxaluria is a common finding (16, 17). According to Starkloff (17), 60% of 300 postoperative patients had elevated urinary oxalate levels, which may be caused by increased absorption of exogenous oxalate, possibly due to decreased calcium binding in the gut. Six percent of Starkloff's preoperative patients developed renal calculi. The preventive regimen he prefers is low oxalate diet with high calcium intake to increase intestinal oxalate binding.

### *Gout*

Although transitory hyperuricemia is common after bypass, clinical gout is rare (3, 17). Nine of 150 patients had gout that required treatment before operation. Three new cases have developed since operation. Amelioration of gouty attacks has occurred in four of the older cases and five are unchanged. One patient who rapidly lost over 200 pounds in an 18 month period developed severe gouty attacks with gouty nephropathy requiring repeated hospitalization for control.

### *Anemia and Vitamin Deficiency*

Persistent iron deficiency anemia with chronic low grade melena has developed since operation in three patients. Repeated endoscopies and X-ray studies of the GI tract have failed to reveal a bleeding site. Response to oral iron has been good.

Serum vitamin concentrations have been measured repeatedly during follow-up in the majority of patients after jejunoileal bypass. Transient deficiencies in the early postoperative months have been observed sporadically in vitamins A, E, folate, and C concentrations in about one third of the patients and have been treated with oral supplements. No chronic avitaminoses have occurred. No evidence of vitamin $B_{12}$ deficiency has developed in any patient of this series.

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# RATIONAL PSYCHOPHARMACOLOGY FOR PATIENTS WITH MEDICAL DISEASES[1]

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## INTRODUCTION

It is no surprise that psychotropic drugs are commonly administered to patients with medical diseases (1, 2). First, nonpsychiatrist physicians often encounter emotional discomfort in their patients with medical diseases. Surveys performed in the Unites States (3–7) and elsewhere (8–10) suggest that a high percentage of ambulatory individuals with medical disorders receive psychotropic drugs because of anxiety or insomnia. Studies of drug dispensing patterns (9, 11, 12) indicate that the majority of psychotropic drug prescriptions are written by general practitioners, internists, and surgeons. Well over 50% of hospitalized medical patients receive pharmacotherapy for anxiety or insomnia (13–15). Second, a number of individuals with primary emotional disorders—particularly the elderly—have coincident medical diseases. Finally, in some medical disorders or syndromes, such as intractable hiccups or severe nausea and vomiting, a psychotropic drug (i.e. a phenothiazine) may constitute a treatment of choice (16).

This pharmacologic interface of medicine and psychiatry is of considerable importance. Informed clinicians require an understanding of whether psychotropic drugs can provide symptomatic benefit for their medically ill patients with emotional distress. They should also understand whether the psychotropic drugs they administer can influence the clinical course of the medical disease and, conversely, whether the response to the psychotropic agent can be affected by characteristics of the patient and the disease state. This article focuses upon such considerations.

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## CLASSIFICATION OF PSYCHOTROPIC DRUGS

Psychotropic drugs are a heterogenous group of pharmacologic agents (16–23). Several classes of psychotropic drugs are in wide clinical use (Table 1). The primary pharmacologic properties, clinical actions, and side effects of these classes of drugs are quite different, and each can interact with medical disease and with other medical therapies in very different ways.

### *Sedative-Hypnotics*

These include the *hypnotic* agents (sleep-inducing agents or soporifics) and the *sedatives* (antianxiety agents, anxiolytics, or "minor" tranquilizers). Hypnotics are used to treat sleep disorders (17, 23–27). Most of these disorders are described as "insomnia" and are characterized by the patient's inability to fall asleep rapidly, to stay asleep, to sleep for an adequate length of time, or to awaken feeling properly rested. Thus in most cases hypnotics are given to simply induce and maintain sleep.

Table 1 Psychotropic drugs in clinical use

| Hypnotics | Antianxiety agents (sedatives, minor tranquilizers) | Antipsychotic drugs (neuroleptics, major tranquilizers) | Tricyclic antidepressants | Antimanic drugs |
|---|---|---|---|---|
| Barbiturates | Barbiturates | Phenothiazines | Dibenzazepines | Lithium Salts |
| pentobarbital | amobarbital | *Aliphatics* | imipramine | lithium carbonate |
| secobarbital | butabarbital | chlorpromazine | desipramine | |
| amobarbital | phenobarbital | promazine[c] | clomipramine[d] | |
| butabarbital | Propanediols | promethazine[c] | Dibenzocycloheptenes | |
| phenobarbital | meprobamate | triflupromazine | amitriptyline | |
| Tuinal[a] | tybamate | *Piperidines* | nortriptyline | |
| Chloral Derivatives | phenaglycodol | mesoridazine | protriptyline | |
| chloral hydrate | Benzodiazepines | piperacetazine | Dibenzoxepins | |
| chloral betaine | chlordiazepoxide | thioridazine | doxepin | |
| trichloroethyl phosphate | diazepam | *Piperazines* | | |
| dichloralphenazone | oxazepam | acetophenazine | | |
| Piperidinediones | clorazepate | butaperazine | | |
| glutethimide | lorazepam[d] | carphenazine | | |
| methyprylon | medazepam[d] | fluphenazine | | |
| Acetylinic Alcohols | bromazepam[d] | perphenazine | | |
| ethchlorvynol | Antihistamines | prochlorperazine | | |
| Quinazolines | hydroxyzine | trifluoperazine | | |
| methaqualone | | Butyrophenones | | |
| Mandrax[b, d] | | haloperidol | | |
| Benzodiazepines | | trifluperidol[d] | | |
| flurazepam | | Thioxanthenes | | |
| nitrazepam[d] | | chlorprothixene | | |
| Antihistamines | | thiothixene | | |
| diphenhydramine | | flupenthixol[d] | | |
| hydroxyzine | | Dihydroindolones | | |
| | | molindone | | |
| | | Dibenzodiazepines | | |
| | | loxapine | | |
| | | clozapine[d] | | |

[a] Combination of amobarbital and secobarbital.
[b] Combination of methaqualone and diphenhydramine.
[c] Weak antipsychotic properties.
[d] Not available in the United States.

By contrast, sedative agents are given to treat the extremely common but ill-defined and evanescent syndrome of anxiety—without producing any symptoms of drowsiness, lethargy, or other manifestations of central nervous system (CNS) depression (18, 22, 24, 28). Not surprisingly, short-term drug therapy of insomnia is usually highly successful regardless of which hypnotic drug is chosen, whereas the clinical efficacy of antianxiety agents can be very difficult to demonstrate.

Although the clinical objectives of hypnotic and antianxiety drug therapy are very different, the pharmacologic properties of these two groups of drugs are very similar or identical. In fact, the same drug (i.e. phenobarbital, diazepam) can serve as a nighttime hypnotic in high doses and as a daytime sedative in low doses (22, 24, 29). All sedative-hypnotics have dose-dependent nonspecific CNS depressant properties. They have no specific effect on the autonomic or peripheral nervous systems, nor any particular influence on organ system functions outside of the CNS. All sedative-hypnotics have anticonvulsant properties, and all have some potential to produce tolerance, habituation, and physiologic addiction.

### *Antipsychotic Drugs*

The antipsychotic agents (neuroleptics or "major" tranquilizers) have the property of reversing many aspects of the disordered thought process of schizophrenia (16, 30). Five groups of compounds comprise the class of antipsychotic agents (Table 1), the most familiar of which are the phenothiazines and butyrophenones. All major tranquilizers have similar clinical efficacy as "antidotes" to schizophrenia (30), currently viewed as reflecting their ability to block dopamine receptors in the CNS (31–36).

Unlike the sedative-hypnotics, the major tranquilizers have a wide variety of additional pharmacologic effects involving central and peripheral adrenergic and cholinergic transmission as well as the physiologic function of almost every major organ system. Some of these, i.e. the antiemetic properties, are exploited for therapeutic purposes, but most of the secondary properties of the major tranquilizers are considered unwanted and labeled as side effects. The most familiar of these include extrapyramidal motor disorders, postural hypotension, impairment of temperature regulation, peripheral cholinergic blockade, and hepatotoxicity (37). A clinician's decision to administer a major tranquilizer and the particular choice of drug should reflect consideration of the patient's ability to tolerate the possible side effects of the drug as well as the potential benefits of therapy.

### *Antidepressants*

*Tricyclics* and *monoamine oxidase inhibitors* (MAOI) are the two major categories of antidepressant agents (19). Because MAOI are more toxic and no more effective than tricyclics, they are seldom indicated for initial pharmacotherapy of depression.

Antidepressant drugs are of considerable value for depressive syndromes associated with "endogenous" depressive illness, but less effective for depressions of the reactive (situational), characterological, or neurotic types (38). Recent studies suggest that tricyclics may also be of value in certain patients with phobic anxiety states and spontaneous panic attacks (19, 28). Tricyclic antidepressants must be given in adequate dosage—at least 150 mg per day of imipramine or its equivalent for most patients with significant affective illness (39). There is also a lag period of up to four weeks from the beginning of therapy until the drugs become effective (40). The anticholinergic effects of the tricyclic antidepressants are of considerable clinical importance, particularly for some patients with certain other medical diseases. Some tricyclics (i.e. amitriptyline) produce nonspecific sedation, while others (i.e. nortriptyline) do not.

### *Antimanic Drugs*

Lithium carbonate is the most useful drug available for the treatment of manic and hypomanic phases of manic-depressive disease ("bipolar" affective disorder) (41, 41). Recent studies suggest that lithium may also be indicated for therapy of certain categories of recurrent "unipolar" depression (43). The toxic-to-therapeutic ratio for lithium is very narrow. To insure safe and effective lithium therapy, dosage should be adjusted to keep serum lithium concentrations in the usual therapeutic range of 0.7–1.5 meq per liter. Troublesome and sometimes serious side effects can appear when serum concentrations exceed this range only slightly. Unlike other psychotropic drugs, lithium ions are essentially unbound to plasma proteins and are excreted unchanged by the kidney.

## PSYCHOTROPIC DRUGS IN MEDICAL DISEASES: A RATIONAL APPROACH

Even the most astute and careful clinicians often fail to invoke their usual decision-making processes when they approach emotional symptoms in medical patients. As a result, ill-conceived and poorly planned drug therapy of these disorders is commonplace. Ideally, nonpsychiatrist physicians should approach emotional symptoms as they would any other kind of symptom, asking themselves the following questions before initiating treatment.

### *Is the Symptom Attributable to an Organic Cause?*

Emotional or psychiatric disturbances in medical patients are frequently due to causes related to the underlying disease. In such cases removal of the underlying etiology is certainly preferable to treatment of the symptom. Common causes of insomnia, anxiety, or agitation in medical patients include alcohol withdrawal, hypoxia, hypoglycemia, pain, paroxysmal tachyarrhythmias, pheochromocytoma, pulmonary congestion, thyrotoxicosis, and drug toxicity (17, 25). Specific therapy is available for essentially all of these conditions. Purely symptomatic treatment with a psychotropic drug, on the other hand, is seldom rational and may actually do harm if anxiety and insomnia are due to hypoxia, for example. Depression of mood and affect can also be due to underlying causes, such as endocrinopathies, uremia, anemia, carcinomatosis, and toxicity from a number of drugs (i.e. reserpine, methyldopa, clonidine, propranolol, and diazepam) (19). Again, treatment or removal of the underlying cause is rational, whereas symptomatic therapy with an antidepressant drug is not.

### *Will the Drug Help the Patient?*

Assuming that no specific organic cause for an emotional symptom can be located, clinicians should then weigh the potential benefits and hazards of psychotropic drug treatment.

Short-term, well-supervised pharmacotherapy of insomnia in medically ill patients has a high likelihood of success with relatively few hazards (26, 44–51). This is especially true in the hospital setting, where the medical staff has control over what drug is taken, its dosage, and the frequency of administration. Among ambulatory patients the hazards of hypnotic drug therapy are somewhat greater since the patient, not the physician, has control of dosage and frequency of administration (52). Furthermore, the potential consequences of residual drug effects or "hangover" are greater for individuals who must perform "real-life" tasks such as operating an automobile (53). Still, short-term drug therapy of insomnia in outpatients can be rational, provided the drug and its dosage are well chosen, small numbers of pills are provided, physician follow-up is frequent, and patients are warned of possible impairment of psychomotor function.

Inadequate dosage explains many apparent failures of hypnotic drug therapy. Doses at the "low" end of the usual recommended range (i.e. 50–100 mg of secobarbital, 500 mg of chloral hydrate, 15 mg of flurazepam) are conservative and will not be adequate for many patients. Many studies show that the likelihood of successful pharmacotherapy is increased considerably when higher doses are used (i.e. 200 mg of secobarbital, 1.0–1.5 g of chloral hydrate, 30 mg of flurazepam), whereas the frequency of untoward effects is increased only slightly if at all (24, 26). Therapy should usually be initiated with these higher doses unless patients are elderly, severely debilitated, or another specific reason exists for using smaller doses.

The success rate of anxiolytic therapy in medical patients is also high, particularly when benzodiazepine derivatives are administered (22). Numerous controlled studies have documented the symptomatic benefit of antianxiety agents in patients with anxiety associated with cardiovascular, gastrointestinal, dermatologic, or gynecologic disorders (22, 54–60). The efficacy of anxiolytic drug therapy in these disease states is approximately the same as in the treatment of anxious individuals without organic disease. Because most controlled trials have been performed in ambulatory individuals, the efficacy of the drugs in hospitalized medical patients is not adequately established.

The pharmacologic effects of the drugs themselves explain only part of their clinical value. Placebo effects, the attention and reassurance of medical staff, the patient's adaptation to his disease, and the tendency for anxiety symptoms to remit spontaneously all contribute to the apparent efficacy of drug treatment in these individuals (22). The need to titrate pharmacotherapy to the needs of the individual patient has been emphasized previously (18). Patients require the drugs most when their emotional discomfort is most severe. Dosage can be adjusted upward at such times and reduced during apparent remissions. Some patients can make these dosage adjustments by themselves. Understanding of the pharmacokinetic properties of antianxiety agents is also of considerable importance for the clinician (61, 62).

Chlordiazepoxide and diazepam are "long-acting" drugs with pharmacologically active metabolites. The parent drugs, their metabolic products, or both can persist in the blood for many days even after a single dose and can accumulate in the body when multiple doses are given. A once- or twice-daily dosage of such drugs is usually sufficient to maintain clinically effective concentrations in blood. For patients whose anxiety is associated with insomnia, most or all of the daily dose can be given at bedtime. The same is true for clorazepate, which is hydrolyzed in the stomach to desmethyldiazepam, a long-acting pharmacologically active metabolite of diazepam. Oxazepam, another benzodiazepine, and meprobamate, a propanediol derivative, have a shorter duration of action and no active metabolic products. Two or more daily doses of these drugs are usually required to maintain effective concentrations.

Manifestations of schizophrenia, such as hallucinations, delusions, inappropriate or labile affect, defective reality testing, or psychotic panic or terror, will occasionally appear in a medical patient with no prior history of similar psychopathology (25). An underlying organic cause can almost always be identified (25). Possibilities include the primary disease process or a complication thereof (i.e. alcohol withdrawal, systemic lupus erythematosus, Cushing's syndrome, acute intermittent porphyria, metastatic carcinoma, or following cardiac surgery or childbirth); a metabolic disturbance such as hypercalcemia or hyponatremia; drug therapy (i.e. corticosteroids, isoniazid, or cycloserine); the patient's environment (i.e. the coronary care unit); or some combination of these factors. Treatment is best directed at correcting the underlying cause, but occasionally short-term pharmacotherapy with a neuroleptic agent may be required. Clinical experience suggests that antipsychotic drugs will ameliorate psychopathology in the majority of such patients, although adequate documentation of this in controlled studies is lacking. The decision to use a drug of this type should reflect careful consideration of the potential risks and benefits, since neuroleptic therapy is not without hazard. The potential adverse effects of the drugs themselves are discussed above and elsewhere (16, 37). Moreover, the use of an antipsychotic drug for symptomatic therapy incurs the risk of masking CNS signs upon which a change in medical therapy might depend.

Depression in medical patients usually constitutes an appropriate response to organic disease (25). For such patients their losses—of bodily integrity, health, and vigor—are real; hence their sadness, pessimism, despair, and despondency are part of the normal grieving process. Such reactive or situational depressions tend to be self-limited. As patients confront and accept their disease, the disabilities or limitations in life-style that they face, and the possibility of a shortened life span, symptoms begin to remit and recovery commences. Many patients find that their initial fears and visions of invalidism were excessive, and they may return to a relatively normal life.

Antidepressant drugs are seldom indicated for medical patients with no prior history of depression whose symptoms appear to be precipitated and perpetuated by medical illness. It is doubtful whether antidepressants are more effective than placebos in reactive or situational depressions (38). More appropriate is a nonpharmacologic approach in which medical staff discusses with the patient his or her concerns and feelings, answers his questions objectively and honestly, and generally provides support while the patient works through his grief. In selected patients a trial of antidepressants may be warranted, as in individuals with a history of depressive illness or in those whose emotional symptoms appear to be inappropriately severe or prolonged. It is not uncommon for an individual with a history of recurrent unipolar depression to have a depressive episode precipitated by medical illness.

### *Will a Psychotropic Drug Influence the Medical Disease or the Response to Medical Therapy?*

This question is important and often difficult for the clinician to answer, because the possibilities for interactions of psychotropic drugs with medical diseases or other medical therapies are so numerous (1, 63). For most sedative-hypnotic drugs the situation is less complicated, since their major pharmacologic effects are attributable to dose-dependent CNS depression. On the other hand, neuroleptics and antidepressants influence many organ systems and can participate in many interactions (63). A discussion of some of these interactions follows in the next section.

## USE OF PSYCHOTROPIC DRUGS IN SPECIFIC DISEASE STATES

A great deal has been published on this subject, mostly in the realm of clinical impression and anecdotal observation. Research in the area is active and results of controlled studies and epidemiologic surveys are increasingly available. This section reviews some factors to be considered when psychotropic drugs are used in specific disease states.

### *Ischemic Heart Disease*

The possible cardiotoxic effects of certain classes of psychotropic drugs are of concern when they are administered to patients with ischemic heart disease. Phenothiazines and tricyclic antidepressants have both been implicated (64). Sudden unexplained deaths have been reported among previously healthy individuals receiving phenothiazines (65–68). Possible causes include profound hypotension, severe hypo- or hyperthermia, or ventricular tachyarrhythmia. The last explanation seems most likely since a number of experimental and pathological studies reveal cardiotoxic effects of phenothiazines (69–71). In several anecdotal reports, life-threatening tachyarrhythmias were attributed to administration of thioridazine (72–76). Usual therapeutic doses of thioridazine and possibly other phenothiazines produce ST- and T-wave changes on the electrocardiogram that resemble hypokalemia and are reversible upon administration of potassium (64, 77–82). These changes may or may not relate to the possible association of such drugs with life-threatening arrhythmias.

Conduction disturbances, tachyarrhythmias, or both commonly occur in patients who have ingested overdoses of tricyclic antidepressants (83–86). Theraupeutic doses can also produce such changes (87–91), but it is not established how frequently they occur, nor whether they constitute a clinically significant hazard to the patient.

Some epidemiologic studies suggest that tricyclic antidepressants increase the risk of sudden death in patients with cardiac disease (92, 93); other studies do not (94). Whether antidepressants interact with concurrently administered antiarrhythmic drugs is not known.

Until more conclusive data are available, clinicians should be aware that antidepressants and phenothiazines might be hazardous in patients with cardiac disease, particularly those with a history of arrhythmias. This is a possible hazard that must be weighed against the potential clinical benefits from treatment with such drugs.

### *Hypertension*

Many clinicians prescribe antianxiety agents rather than specific antihypertensive drugs for their patients with hypertension (1). This unfortunate practice usually results in a drowsy but still hypertensive patient. Antianxiety agents have no specific hypotensive effect; when they lower blood pressure it is due to CNS depression. In most patients anxiolytic drugs produce clinically important reduction in blood pressure only at doses that produce an unacceptable degree of sedation. Antianxiety agents may be of benefit when given adjunctively with specific antihypertensive drugs in patients whose anxiety is judged to contribute to blood pressure elevation, but verification of this impression in controlled studies is lacking. Barbiturates probably should not be administered because of their enzyme-inducing properties. In one study barbiturate administration appeared to accelerate the metabolism of methyldopa (95), but another group of investigators did not confirm this (96). In an animal study phenobarbital stimulated the metabolism of propranolol (97), a $\beta$-adrenergic blocker often used to treat hypertension.

Guanethidine sulfate is a widely prescribed antihypertensive agent (98, 99). Several clinical reports show that its hypertensive effect is partially or completely antagonized by coadministration of a tricyclic antidepressant (100–103). The antagonism develops only after at least 12 hr elapse following initial administration of tricyclic drugs (104, 105). Doxepin is a less potent antagonist than other antidepressants, producing guanethidine reversal only at relatively high doses (106, 107). Recently a similar antagonistic effect has been attributed to chlorpromazine (108). Antidepressants appear to prevent guanethidine from reaching its site of action within the presynaptic nerve terminal (103). Antidepressants also inhibit the action of bethanidine (103) and clonidine (109), two other antihypertensive drugs. Concurrent therapy with guanethidine and tricyclic antidepressants is clearly not rational.

### *Seizure Disorders*

Major tranquilizers and tricyclic antidepressants can probably lower the seizure threshold and precipitate clinical seizure activity in susceptible individuals (110–115). Predicting who is at risk of this complication is difficult, particularly since some patients will have fewer seizures when they are less anxious or agitated. In clinical reports most patients whose seizures were attributed to these drugs had no prior history of a seizure disorder. One study suggested that grand mal convulsions occurred more frequently among phenothiazine-treated patients than among untreated "controls" (116). The frequency increased with dosage. The implications of this study are unclear, since it was retrospective and allocation of patients to treatment groups was not random.

Because reliable data are lacking, the frequency and clinical significance of seizures induced by therapeutic doses of major tranquilizers and tricyclic antidepressants is not established. Hundreds of thousands of patients, many of whom are receiving concurrent therapy with anticonvulsant drugs, are treated with these psychotropic agents with no evidence of either new seizure activity or reduced seizure control. One exception is the treatment of alcohol withdrawal. Several well-controlled trials show that withdrawing alcoholics treated with phenothiazines have seizures more frequently than those receiving placebos or other sedative-hypnotics (such as paraldehyde or chlordiazepoxide) (117–120). The data are convincing enough to suggest that major tranquilizers should not be used to treat the alcohol withdrawal syndrome, especially when patients have an underlying seizure disorder (121, 122).

## *Pulmonary Disease*

Legitimate concern exists about the administration of CNS depressant drugs to patients with obstructive pulmonary disease. Respiratory failure is a dramatic and life-threatening event, and clinicians do not readily forget occasions on which psychotropic drugs appeared to precipitate it. Unfortunately, such memorable anecdotes tend to supersede rational assessment of the hazards of psychotropic drug use in patients with impairment of the respiratory drive. The influence of anecdotal evidence (123, 124) is reinforced by studies of pulmonary function (125–129) that demonstrate statistically significant changes in the direction of hypoxia, hypercapnia, and acidosis associated with administration of benzodiazepine derivatives, for example. Such reports are quoted as demonstrating "respiratory depression," but close scrutiny reveals that the *magnitudes* of the reported changes in most of these studies are small and of very uncertain clinical significance. Caution is always warranted when psychotropic drugs are administered—particularly intravenously—to patients with impairment of the respiratory drive. The actual hazards, however, are probably exaggerated (128, 130–133); very little data are available regarding how frequently such drugs produce clinically important respiratory depression.

Anxiolytic agents are included with xanthine derivatives and sympathomimetic amines in a number of oral antiasthmatic combination preparations. Presumably, the purpose of the sedative drug is to counteract the CNS stimulant effects of the two other drugs and to treat the emotional component of the disease. There is little documentation of whether the anxiolytic drug adds any clinical benefit to that attributable to the two bronchodilator constituents (22). When phenobarbital is the sedative component it may have an adverse clinical effect. In one study of corticosteroid-treated asthmatic patients, phenobarbital administration stimulated hepatic metabolism of the steroid and antagonized its clinical effect (134).

## *Gastrointestinal Disorders*

The rate of drug absorption from the gastrointestinal tract depends in part upon the rate of gastric emptying. Pentobarbital absorption is delayed when the drug is given with food (135). Coadministration of chlorpromazine with antacid preparations reduces the absorption rate and might also reduce the completeness of absorption (136, 137). Chlordiazepoxide absorption is delayed when given together with Maalox® (D. J. Greenblatt and R. I. Shader, unpublished observations). These findings are relevant to the clinical use of psychotropic drugs in patients with peptic ulcers. Little data are available on the absorption of psychotropic agents in malabsorption syndromes.

Since tricyclic antidepressants have significant atropine-like properties they can reduce gastrointestinal motility. Absorption of phenylbutazone (138) and L-dopa (139) is delayed when coadministered with antidepressants. The cholinergic blocking properties of antidepressants as well as neuroleptics may be potentially hazardous in patients in whom gastric or bowel obstruction is present or impending (37).

Hepatocellular dysfunction can alter the hepatic metabolism of a number of drugs. This has been reported in patients with hepatitis or cirrhosis (140–143). Some studies suggest that liver disease must be severe enough to result in hypoalbuminemia before drug clearance is significantly impaired (144–146). There are relatively few studies with psychotropic drugs. In one report chlorpromazine clearance was not significantly impaired in patients with cirrhosis, nor were its central depressant effects potentiated (147). Other studies demonstrate significant impairment of diazepam (148) and barbiturate (146, 149) clearance in hepatocellular disorders. An epidemiologic survey suggested that drowsiness due to chlordiazepoxide and diazepam was more common in patients with hypoalbuminemia (150). Caution appears to be warranted when psychotropic drugs are administered to individuals with liver disease, since the clearance rate of the drugs may be reduced. Also of concern is the possible precipitation or potentiation of hepatic coma. One study showed that small doses of diazepam could be safely administered to patients with cirrhosis (151).

A popular yet totally unfounded myth suggests that antihistamines (i.e. diphenhydramine) and paraldehyde are "safer" than other psychotropic drugs for patients with liver disease. On the contrary, both of these drugs are metabolized by the liver (152–154), and only a small percentage of a dose of paraldehyde is exhaled via the lungs (154). Thus antihistamines and paraldehyde are as "hazardous" as other psychotropic drugs in such patients.

### *Thromboembolic Diseases*

Drug interactions during oral anticoagulant therapy has received much attention (24, 155). The enzyme-inducing effects of barbiturates and glutethimide are well known and can result in clinically important antagonism of oral anticoagulant drugs (24, 155). Benzodiazepines do not seem to share this enzyme-inducing property and can be safely administered to patients receiving anticoagulants (22). Chloral derivatives cause transient potentiation of coumarin anticoagulant effects because trichloroacetic acid, a major metabolite of chloral derivates, displaces coumarin drugs from their binding sites on plasma protein (156, 157). For the same reason, patients taking chloral hydrate require lower doses of anticoagulants during initiation of therapy than do patients not receiving chloral hydrate (158, 159). One study suggested that nortriptyline delayed the metabolism of bishydroxycoumarin in man

(160). In another report tricyclics had no influence on the metabolism of warfarin (161). There are no reported interactions of psychotropic drugs with heparin.

## CONCLUSION

Psychotropic drug treatment in medically ill patients should not be undertaken lightly. Emotional or psychiatric symptoms should be evaluated as carefully as any other sign or symptom in such patients. Initiation of psychotropic drug therapy should follow the same assessment of risks and benefits as would treatment with any other class of drug. Safe and effective psychotropic drug use is more likely when it is integrated into the overall scheme of medical management.

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# MEDICAL ASPECTS OF RIOT CONTROL (HARASSING) AGENTS ❖7211

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## INTRODUCTION

Events of the past decade have resulted in the increased use of tear gas devices. "Tear gas" is a misnomer applied to a number of chemicals, dispersed as aerosols, that have been classified as "harassing agents" (1). Harassing agents are defined as chemical agents that are capable, when used in field concentrations, of rapidly causing a temporary disablement that lasts for little longer than the period of exposure (2). They are distinguished from such incapacitating agents as the psychochemicals in that the psychochemicals have a delayed onset of action and the effects persist for a period greatly exceeding that of exposure. A distinction between these two classes of chemical agents may also be drawn on physiological grounds: harassing agents are sensory irritants and psychochemicals are centrally acting substances.

## HISTORICAL BACKGROUND

While poisonous gas produced by burning wax, pitch, and sulfur was used as early as 428 BC during wars between the Athenians and the Spartans, chemical agents that temporarily incapacitate by producing lacrimation and uncontrollable blepharospasm were first developed and used by the German Army in World War I (3, 4). Smaller purse or pen-sized tear gas weapons became available over the counter for personal protective use in the early 1920s (4). Around 1965, pressurized dispensers (liquid stream or aerosol) became available over the counter (5). One of the approximately 25 commercial preparations of tear gas, known by the trade name Chemical Mace® (General Ordnance Equipment Company), has achieved rather ignominious fame; however, few persons, including physicians, recognize that Chemical Mace is not a new material, but rather, a common tear gas (chloroacetophenone) marketed in a pressurized aerosol canister.

The increased usage of harassing agents has not been accompanied by general dissemination of information to physicians, who should be knowledgeable about the nature of these substances and their effects, since individuals may seek medical attention after exposure, especially if they have incurred concomitant injuries. In the interval from June 1968 through June 1971, the Department of Human Resources of the District of Columbia reported that more than 600 individuals were treated for tear gas effects (6). It is the purpose of this review to summarize the material that has been published regarding the harassing agents to which civilians might be accidentally or intentionally exposed.

## CHEMISTRY

Some 15 sensory irritants have been used on occasion; however, only four agents —CN, CS, DM, and BBC (α-bromo-α-tolunitrile)—have been used extensively (2). The properties of the three most commonly employed agents are summarized in Table 1 and their structures presented in Figure 1. Of these agents, only CN and CS have been widely employed in the United States, the United Kingdom, and Europe. In the United States all but one of the over-the-counter products contain CN. The derivation of the designation CN is unknown. The designation CS is derived from the names of the two chemists (Corson and Stoughton) who isolated the compound in 1928 during a study of unsaturated nitriles (7). DM is derived from its name, adamsite.

### *Biochemical Interactions and Metabolic Fate*

Both CN and CS are $S_N2$ alkylating agents that react with sulfhydryl (SH) groups (nitrogen mustard is an $S_N1$ type alkylating agent that initially reacts with water to form active carbonium ions; in contrast, $S_N2$ agents do not require a primary reaction with water) (8). CS reversibly inhibits lactate dehydrogenase, an SH-dependent enzyme, in vitro while CN does not (8). Pharmacologic studies have

Table 1 Properties of selected harassing agents

| | Common name | | |
|---|---|---|---|
| | CN | CS | DM |
| Chemical composition | w-chloroacetophenone (phenacyl chloride, phenyl chloromethyl ketone) | *ortho*-chlorobenzylidene malononitrile | 10-chloro,5,10-dihydrophenarsazine |
| Form in which usually disseminated | aerosol or dust | aerosol or dust | aerosol or dust |
| Physical state at 20°C | solid | solid | solid |
| Solubility in water at 20°C | 105 mg/m$^3$ | negligible | 0.02 mg/m$^3$ |
| Dosage to produce incapacitation | 5-20 mg/m$^3$ | 1-5 mg/m$^3$ | 2-5 mg/m$^3$ |
| Estimated human respiratory lethal concentration$_{50}$ | 11,000 mg-min/m$^3$ [a] | 25,000-150,000 mg-min/m$^3$ | 15,000 mg-min/m$^3$ |
| Characterization by predominant response | lacrymator | lacrymator | sternutator[b]. |
| Potential for skin sensitization | + | + | 0 |

[a] Concentration in mg/m$^3$ × min.

[b] An agent which induces the flow of nasal mucus and/or causes sneezing.

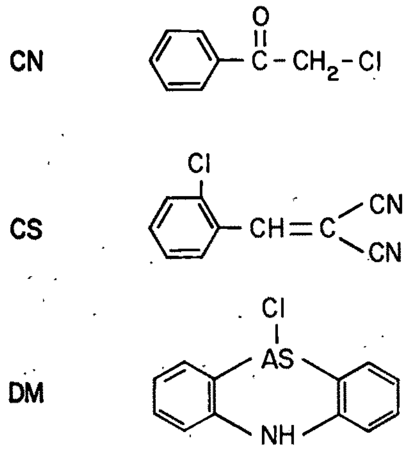

*Figure 1* Structure of selected harassing agents.

primarily involved CS. The symptoms and time of onset of poisoning by CS and malononitrile are similar at equimolar doses (9). Since the toxicity of malononitrile has been ascribed to its conversion to cyanide in tissues, the possibility that the same mechanism of toxicity holds for CS was tested by administration of sodium thiosulfate to animals given lethal doses of CS. Rapid improvement ensued; however, late deaths (several days) occurred, suggesting both a cyanide effect and a second, late form of toxicity (9). A person standing 20–30 m downwind from a 50-g CS cartridge would retain at most 2.1 $\mu$mole of cyanide; an amount comparable to that present in two puffs of a cigarette (10). Dogs exposed to low aerosol concentrations of CS show rises in blood pressure, heart rate, and respiratory rate. CS results in the generation of bradykinin both in vitro and in vivo (8). Following inhalation of toxic aerosol doses by dogs, acidosis, hemoconcentration, and hypothermia develop, with death occurring in two to three days. The major metabolites of CS are *ortho*-chlorobenzyl malononitrile ($CSH_2$) and *ortho*-chlorobenzaldehyde (OCB) (11). These metabolites as well as CS may be absorbed from the respiratory tract into the bloodstream in cats exposed to 100 mg/m$^3$ for 5 min (12). Absorption of metabolites of CS may occur from the gastrointestinal tract (10). Both CS and its metabolites are rapidly cleared from the circulation; the $t_{1/2}$ being 4.5–9.5 sec (12). Extrapolation to man suggests that CS and its metabolites could be absorbed from both the respiratory and gastrointestinal tract; however, since the maximal tolerable concentration of CS in man is approximately 10 mg/m$^3$, it is improbable that significant amounts would be absorbed (12).

## DELIVERY DEVICES

Since CN, CS, and DM are relatively insoluble in aqueous solutions and nonvolatile at ambient or body temperatures, their usage depends upon delivery of aerosols that will contact susceptible nerve endings. To achieve this, two general types of tear gas devices, the pressurized type and explosive cartridge type, are employed. Both types generate particles of 0.5–2 μm mass mean diameter. Aerosols may be produced pyrotechnically by grenades in which CS is mixed with a pyrotechnic composition. With detonation the CS is volatilized, then recondenses into aerosols. On a calm day the usual grenade would generate a cloud 20–30 ft in diameter, which may linger for 10–15 min (13). A concentration of 2000–5000 mg/m$^3$ may be produced at the point of burst (13). Until recently small explosive cartridges, usually with CN sealed into a cardboard container with a wadding of cork, wax, or rubber and a detonator charge similar to a shotgun shell, were most commonly employed. In commercial products of the pressurized type, kerosene, 1,1,1-trichlorethane, and trichlorotrifluoroethane (Freon 113) are common constituents (5). In the United States, a group of commercial tear gas devices was tested and found to contain 0.04–4.3% wt/vol CN with only 3 of 25 preparations containing >0.1% CN(5). Only one commercial spray with CS was found (5). In contrast, in the UK, CS is employed almost exclusively because it is approximately 10 times more potent than CN as an irritant and at the same time is less toxic (14).

## CLINICAL FEATURES

The acute transient clinical effects of both CN and CS are quite similar and hence are considered together. It should be recognized that most of the data have been derived from studies on CS. Following exposure of volunteers to CS aerosols with mass mean diameters of 1.0 μm at concentrations of 10–40 mg/m$^3$, response times ranged from 7–60 sec (15). Hyperventilating subjects, i.e. those running 100 yards, could not tolerate CS as well. Their recovery time after terminating exposure was slightly prolonged to 1–2 min. Also, at higher ambient temperatures (95°F) and higher relative humidity (97%), CS was less well tolerated (15). Major symptoms involve the eyes and respiratory tract (15, 16). Instantaneous conjunctivitis, accompanied by burning and pain that persists 2–5 min and usually disappears abruptly, is characteristic. Concomitant blepharospasm is common. Conjunctivitis remains intense for 25–30 min. Erythema of the eyelids occurs and may persist for an hour. Profuse lacrimation invariably occurs and continues for 12–15 min. Photophobia occurs in 5–10% of subjects. Respiratory symptoms include a stinging sensation in the nose and mouth; rhinorrhea; excessive salivation; irritation in the throat; and a burning sensation in the chest associated with tightness, coughing, and occasionally dyspnea. Other symptoms include nausea in some individuals, which rarely is accompanied by vomiting; irritation (stinging) of the skin in the facial region (especially in shaved areas), neck and ears; occasionally a burning sensation of the external genitalia, especially during micturition; and occasional headache. All symptoms usually clear within 10 min, although some features may persist. Physical examination of volunteers during exposure revealed a mild increase in blood pressure but no changes other than noted above. Pulmonary rales did not develop. Hematological studies, including hemoglobin and total and differential leucocyte counts, revealed no abnormalities (16). Blood chemistries revealed increases in total serum protein and bicarbonate concentrations over control values, although both elevated values remained within normal range. Electrocardiograms were normal, as were chest roentgenograms and ventilatory function studies (peak flow rates, tidal volumes, and vital capacities).

At low aerosol concentrations, within 2–3 min following exposure, DM causes severe irritation to the upper respiratory tract and the eyes (2). Irritation begins as a tickling sensation in the nose followed by sneezing and rhinorrhea. Irritation spreads to involve the throat with coughing and choking. Severe headache, especially frontal, occurs and there is often a feeling of pressure in the ears and pain in the jaws and teeth. These symptoms are accompanied by an oppressive pain in the chest, dyspnea, nausea (usually associated with retching and vomiting), a feeling of unsteadiness, vertigo, and trembling. Mental depression may occur. Recovery is usually complete in 1–2 hr (2).

### *Injury from Tear Gas and Tear Gas Weapons*

MacLeod summarized 16 published reports that included 115 to 120 patients who sustained injuries from tear gas and tear gas weapons, mostly CN (4). The injuries sustained by all but three of the patients were ocular. Other injuries included chemical pneumonia and peripheral nerve injuries.

Most instances of eye injury have been associated with exposure to explosive-type devices discharged close to the face. Under these circumstances it is difficult to determine whether the ocular damage is attributable to the lacrimator or secondarily to the cartridge wadding. In a study of 14 eyes from 13 men, which required enucleation following injury from tear gas weapons, the pathological findings included suppurative necrotizing keratitis, iridocyclitis, retrocorneal membranes, and numerous foreign particles (17). With the utilization of pressure-type devices it appears that the likelihood of ocular injury is less. In experimental animals it has been shown that the occurrence of ocular lesions is determined by the concentration of the agent and duration of exposure. The changes range from conjunctival irritation and edema to corneal edema with involvement of the epithelium, stroma, and endothelium with potential loss of each layer with subsequent vascularization and scarring (17). These lesions usually heal without permanent damage. Repeated exposure to CN and CS may also result in an allergic conjunctivitis and blepharitis (17). From studies in animals it has been shown that CN, but not CS, has the potential for producing long-term ocular damage (5). Following the direct installation of CN into the eyes of anesthetized rabbits and monkeys, corneal opacities could be produced (4). The anesthesia is important because it blocks the blinking reflex. When restrained animals were exposed to an aerosol of CN at a distance of 4 cm, permanent corneal damage occurred; however, when animals were similarly exposed at a distance of 6 ft, permanent damage did not ensue. Gaskins et al determined that iritis and corneal opacities could be induced in rabbits with CN at concentrations of $\leq 10\%$ wt/vol, while even at these concentrations SC produced only transient conjunctivitis (5). Rengstorff & Mershon earlier had found no corneal damage following direct installation of CS into the eyes of rabbits and monkeys (18). In volunteers, installation of 1% CS wt/vol into the conjunctival sac produced transient conjunctivitis but neither early nor late corneal damage. With human exposure under field conditions at $\leq 5.0$ mg/m$^3$ the irritation is so intense that subjects are unable to keep their eyes open even a few seconds; however, several minutes following exposure, visual acuity was normal in all subjects (19).

Almost all individuals exposed to CN or CS will experience some stinging of the skin. Transient erythema of the neck and ears is often seen in Caucasians after exposure to *Ct*'s (*Ct* is concentration of the agent in mg/m$^3$ X time in minutes) of 25–50 in tropical climates, since high heat and high relative humidity increase the permeability of the stratum corneum (13). Exposures to CS with *Ct* of $\leq$14,000 may produce prolonged erythema and bullae on the forearm while on the neck, bullae may develop after *Ct*'s of 1000–3000. There is a racial difference, since blacks are less susceptible to the direct cutaneous effects than Caucasians (13). Experimentally, in animals and man, CS produces less dermal irritation than CN (5, 20).

An individual with prior exposure to CN who developed a generalized vesicular dermatitis 24 hr after reexposure to CN was reported in 1941 (21). Rothberg has demonstrated that guinea pigs can be sensitized both to CN and CS but not to DM by intradermal or topical application (22). Since CS is rapidly bound to plasma proteins, its haptenic potential is not unexpected. Allergic contact sensitivity to CN can also be readily induced in man (21). Specific information on the prevalence of CS dermatitis in US personnel in the Republic of Vietnam is limited; however, two observers specifically investigating problems related to the use of CS neither saw nor heard of severe reactions (13).

Since personal tear gas devices have been primarily of the explosive cartridge design, it is not surprising that instances of accidental hand injury have occurred. Adams, Fee & Kenmore (23) reported three patients with hand injury and nerve damage. The nerve damage may have been due to progressive changes produced by fibrosis or direct chemical injury by the CN.

Fatalities have occurred following intense, prolonged exposure to CN. Cucinell found that at exposures of 57,000 mg –min/m$^3$, 50% of dogs died (8). Of note is the fact that these animals died after 48–72 hr. Six deaths have been reported: each individual was exposed in an enclosed environment to high concentrations of CN for intervals from 10 min to several hours (24). Deaths occurred in 8 hr to four days (24, 25). The cases described in detail are of note in that initially the patients' lungs were clear on auscultation and by X ray (24, 25), with respiratory distress (pulmonary edema) developing after 12–24 hr. There is an anecdotal report indirectly from Vennema noting two deaths among seven individuals presumably exposed to a harassing agent in the Republic of Vietnam (26). The data are insufficient to allow any evaluation.

## TREATMENT

Other than prompt withdrawal from the area in which the aerosol of CN, CS, or DM is present, specific treatment is not required and prompt recovery follows. In the management of the conjunctivitis, topical anesthetics are not recommended (17). Cutaneous reactions should be managed by decontamination with copious amounts of water. Since CS hydrolyzes more rapidly to nonirritating products at pH 9–10, an aqueous solution of 6% $NaHCO_3$ or 3% $Na_2CO_3$ may more promptly relieve stinging (13). If an allergic contact dermatitis occurs, topical corticosteroids may be used and for pruritis, antihistamines or cyproheptadine may be used. Management of potentially lethal exposures should include hospitalization and the close monitoring of pulmonary function, both ventilatory and gas exchange, with attention directed toward late (12–48 hr postexposure) deterioration. Experimentally, the administration of penicillin and isoproterenol had no effect on mortality in animals following massive aerosol exposure (27).

Prevention of secondary exposure of medical personnel through contact with residual aerosols on skin or clothing must be considered. Facilities for the disrobing and showering of patients prior to entry into emergency care facilities should be planned in larger hospitals.

## DELAYED EFFECTS OF DIRECT HUMAN EXPOSURE

There is wide concern regarding the possibility of carcinogenesis, teratogenesis, and mutagenesis associated with CN and CS, especially since both are alkylating agents (2, 28, 29). In older studies whose design can be criticized, CN applied to mouse skin at 15 weekly intervals in concentrations of 0.4–0.8% wt/vol proved to be a promoting agent in the induction of benign papillomas when used after dimethylbenzanthracene (30). CN was not as potent as croton oil in this model. The concern that CS might result in serious late effects has resulted in extensive study, especially in Great Britain, and has been reviewed in two official reports, the so-called Himsworth Committee reports (31, 32). They concluded: "There are at present no experimental indications that long term effects, like cancer, might develop after recovery from the early effects of inhaled CS smoke, but a more definite assessment of the risks, if any, of such occurring must await completion of survival experiments on animals still in progress" (31). The long-term effects of CS on animal lungs still have not been reported. A possible relationship has been noted between mustard gas injury sustained in World War I and the subsequent development of lung cancer (33). In a comparison of lung cancer rates in veterans who had been hospitalized for mustard gas injury with those in controls hospitalized for pneumonia and nonhospitalized controls, there were no significant differences in lung cancer deaths between groups. Using a less direct comparison, i.e. individuals exposed to mustard gas with the overall US statistics for lung cancer, the data are "quite suggestive that an association exists" (27). Chemically, CS is not similar to any known carcinogen and it is not metabolized to form one. People exposed do not develop chromosomal abnormalities. CS was neither lethal nor teratogenic in chick embryos (34). Likewise, in pregnant rats and rabbits CS was neither embryo lethal nor teratogenic and had no effect on the number of implantations or litters produced (34). Abortion, stillbirth, and congenital abnormality records for Londonderry do not suggest an interference with pregnancy (10). Thus the available evidence is essentially negative; however, the soundest view is that of the Himsworth Committee that compounds used in the control of civil disturbances should be subjected to the types of preclinical and clinical studies that are required for drugs prior to licensure, rather than being regarded as weapons that are not usually studied from the standpoint of early and late toxicities.

## CONCLUSION

The increased use of harassing agents by individuals and law enforcement authorities has brought the need for an awareness of the immediate and late effects of exposure to CN and CS to the attention of the clinical investigator, teacher, and practicing physician. This review is an attempt to provide an unemotional summary of the available information.

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# EFFECTIVE USE OF ANALGESIC DRUGS

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## INTRODUCTION

No brief review can cover the multiple aspects of analgesic drug use. Recent reviews bearing on pharmacologic and clinical aspects of these drugs have appeared elsewhere (1–3). The intent of this review is to provide the clinician with a basis for effective use of these drugs in patients, based on recent advances in our knowledge of their actions and uses.

Sources of pain may be classified as (*a*) skin and subcutaneous tissues; (*b*) skeletal muscle, bones, and joints; or (*c*) viscera and their coverings. The source of pain may have a bearing in the selection of treatment for it; all sources of pain may be relieved by nonpharmacologic measures as well as by selected drugs. If reversal of the pathogenetic factor responsible for the pain is not possible, nor its relief by nonpharmacologic means, one may choose either analgesics that work peripherally or those that work centrally. Non-narcotic analgesics work primarily in the periphery; the narcotic analgesics act centrally. The former drugs are most frequently and effectively used for pain originating in the skin, subcutaneous tissues, skeletal muscle, bones, or joints. Drugs acting centrally are most often used for visceral pain or for severe injuries to integument or muscle, such as major burns or crushing injuries.

## NARCOTIC ANALGESICS

### *Chemical Structures*

Long before the structure of morphine had been elucidated, attempts were made to alter its effects by chemical modifications. The first of these, in 1874, resulted in diacetylmorphine (heroin), a drug much in the public awareness today. The structure of morphine was not established until 1925, while awareness of its three-dimensional structure is more recent. Knowledge of the chemical structure has been used to make homologues of morphine that might be better analgesics, either in terms of diminished side effects or lessened liability to create dependence. The search has been long, and in a sense productive, for many semisynthetic or synthetic homologues have been developed that are active, sometimes being as much as 10,000 times as active as morphine itself. The incidental discovery that a presumed morphine antagonist (N-allylnormorphine or nalorphine) also had analgesic activity in man focused attention on a new group of drugs with mixed ratios of agonist and antagonist actions. The culmination of the search for drugs of this type has so far been represented by pentazocine, a far from perfect solution to the separation of analgesia from dependence liability (4).

The naturally occurring phenanthrene alkaloids in opium are morphine and codeine. Their pentacyclic structures are shown in Figure 1. Recognition of the phenyl-N-methylpiperidine moiety as the active site for attachment to receptors has allowed the polycyclic structure of morphine to be simplified, ultimately to the basic moiety itself. Morphinans are derivatives in which the oxygen bridge has been eliminated, making a tetracyclic structure. Benzomorphans are further reduced to tricyclic forms. The first truly synthetic opioid, pethidine (meperidine), represented the further reduction of structure to the phenylpiperidine moiety only. Methadone, while appearing to be a diphenylmethane derivative, can assume a phenylpiperidine structure as drawn, and thus is not an exception to the basic chemical requirements.

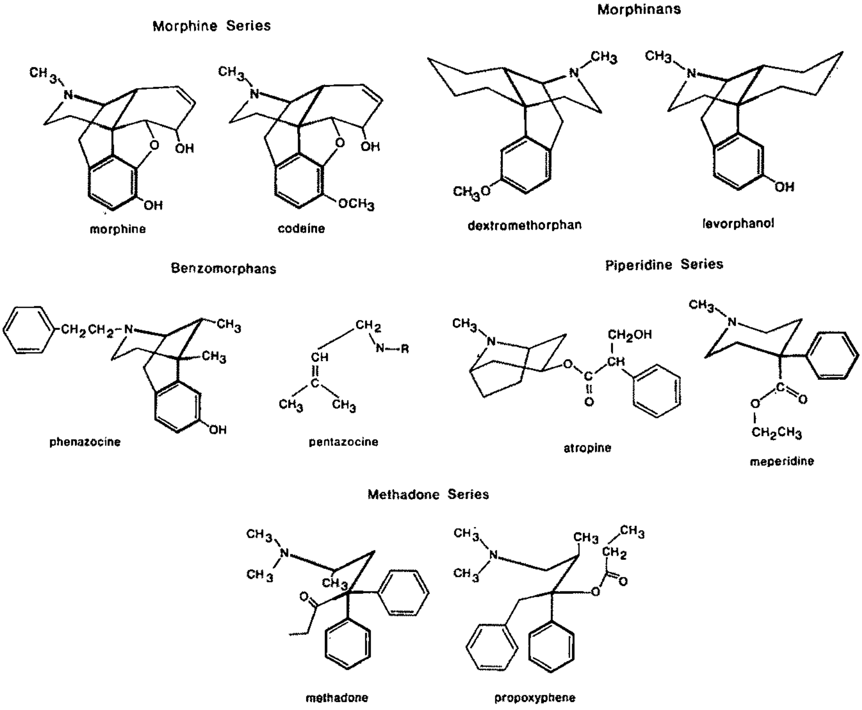

*Figure* 1 Representatives of five different chemical classes of opiates and opioids showing progressively simpler structures. Phenyl-N-methylpiperidyl moiety outlined in heavy ink.

## *Pharmacology and Pharmacokinetics*

Narcotic analgesics act centrally, but until recently there has been little idea of where they may act. During the past few years, a number of laboratories have been actively engaged in searching for opiate receptors. Stereospecific binding of labeled morphine derivatives to nervous tissue has been demonstrated with a number of techniques. Competition for such binding correlates with the in vivo potency of the competing drugs as morphine agonists or antagonists. Such specific binding is widely distributed in the brain, with most sites found in the corpus striatum. Subcellular binding is mostly in the synaptosomal-mitochondrial and microsomal fractions. Whether or not there is a single set of opiate receptors or whether there may be multiple ones, perhaps differentially involved in the multiple effects of the drug, remains to be elucidated (5, 6). Some of the same laboratories that have been investigating the opiate receptor have reported finding ligands for narcotic antagonists in the pituitary as well as in the brain itself. These substances appear, on the basis of early pharmacological testing, to be "endogenous opioids," whose function is not at all clear (7, 8). Nonetheless, the rate of progress in this area of inquiry is proceeding so rapidly that we may soon have a much clearer idea of the site and mode of action of these drugs.

Even so, we may not necessarily learn more from these inquiries about central mechanisms of pain. Commonly, patients treated with opiates report that they can still perceive pain but that it is no longer a source of concern. Thus, these drugs block the "processing" component of pain rather than any specific pain pathway.

Tolerance to narcotic analgesics develops with continued use, requiring that doses be increased to obtain the same effects. Fear of inducing drug dependence has probably led to the situation in which many patients in severe pain are grossly undertreated. Such a fear is largely unfounded, for the number of opiate-dependent individuals initiated by medical treatment is miniscule in relation to the social problem of opiate abuse.

The accurate determination of plasma concentrations of opiate drugs is a fairly recent development. Following intravenous doses of 0.1 mg/kg of morphine sulfate to unpremedicated surgical patients, plasma levels of morphine declined from 240–390 ng/ml at 1–2 min to less than 5 ng/ml at 4 hr, in a biexponential fashion. The fast half-life was 6–16 min with a slow half-life of 2–2.5 hr. Morphine metabolites declined from a maximum of 94–137 ng/ml at 15 min to less than 40 ng/ml at 4 hr with an apparent half-life of 3 hr (9).

After a single rapid intravenous injection of 0.8 mg/kg of pethidine, plasma concentrations declined biexponentially with a fast phase of 11 min and a slow phase of 3.2 hr. In patients with cirrhosis of the liver, the slow-phase half-life was increased to 7 hr, and pethidine clearance was reduced to about one half. Plasma binding of the drug was about 64% in both types of patients, and volumes of distribution were also similar, initially 1.54 l/kg and increasing to 4.17 l/kg at steady state (10). Two metabolites of pethidine, norpethidine, and pethidine-N-oxide have been described, but their functional significance is uncertain. Pethidine readily passes the placenta and could lead to some degree of fetal distress if the drug is used in labor too close to the time of delivery (11). Aside from differences in handling of pethidine by cirrhotics, heavy users of alcohol showed a higher volume of distribution than normals, possibly explaining some of the resistance of alcoholics to central nervous system (CNS) depressants. Increasing age, on the other hand, was associated with increasing fraction of unbound drug in plasma, possibly explaining the greater respiratory depressant effects of these drugs in the elderly (12).

Pentazocine is extensively metabolized, with less than 15% excreted as the parent compound. Oxidation of the two terminal methyl groups on the dimethylallyl side chain of the molecule is the primary pathway. Approximately 30% of the drug is excreted as conjugates of these metabolites (13). Plasma protein binding of pentazocine varies from 48 to 75%, the range being wider in patients than in control subjects. Blood cells contain 48% of the total amount of pentazocine in whole blood, 33% is in plasma proteins, and 19% is in plasma water. Thus, whole-blood concentrations should be roughly equal to plasma concentrations, given a normal hematocrit (14).

Following 130 mg single oral doses of propoxyphene, peak plasma levels were attained in 2 hr followed by a rapid elimination with a half-life of 3 hr. The major metabolite, norpropoxyphene, reached a peak at 4 hr and decayed more slowly (15). The possibility that smoking may contribute to the induction of enzymes for propoxyphene was raised by the observation that the drug was rated as clinically ineffective twice as often in smokers using more than 20 cigarettes daily as in nonsmokers (16).

### *Effects on Specific Systems*

Loss of concern about pain, drowsiness, and euphoria are the predominant effects of narcotics in patients with pain. If the patient is exhausted, sleep may follow after pain is allayed, but hypnosis is not a primary action. Some patients may experience mental confusion or apprehension due to loss of control.

Narcotics depress respiration by decreasing the sensitivity of respiratory chemoreceptors to the stimulus of carbon dioxide, but not to hypoxia (17). Patients with respiratory insufficiency may be precipitated into coma because of carbon dioxide retention. As breathing is sustained mostly by hypoxia, administration of oxygen may result in complete apnea. The tendency to cause carbon dioxide retention with secondary cerebrovascular dilation creates the hazard of aggravating increased intracranial pressure. Relief of the sense of dyspnea with consequent reduction in the work of breathing, in addition to peripheral pooling of blood through increased venous capacitance, accounts for the beneficial effects of morphine in acute pulmonary edema. Suppression of cough reflex, another property of opiates, is exploited primarily by those drugs active orally, such as codeine or dextromethorphan.

Large doses of morphine given intravenously are often used for anesthesia in cardiovascular operations. Such doses given to cardiac surgical patients markedly increase cardiac index, stroke index, central venous pressure, and pulmonary artery pressure, while decreasing peripheral vascular resistance. In the erect position, orthostatic hypotension may be induced due to a direct effect of morphine on the smooth muscle of peripheral vessels.

Nausea and vomiting, two of the major side effects of opiates, are due to stimulation of the emetic chemoreceptor trigger zone; vomiting may also be enhanced by increased labyrinthine sensitivity. Concurrent use of antiemetic or anti-motion sickness drugs may be required to alleviate these unwanted effects. Motility of the bowel is decreased, producing constipation. This effect may be used to good advantage in cases of diarrhea, where formerly camphorated tincture of opium (paregoric) and more recently diphenoxylate, have been used with good effect. Both morphine and pethidine cause spasm of Oddi's sphincter, increasing biliary tract pressure. Pentazocine has been said to do this less, but that may not necessarily be true (18).

Sphincter tone and detrusor urinae muscle activity are increased by opiates, sometimes leading to urinary retention as a postoperative complication. Morphine and codeine in particular, but probably also other opiates, produce histamine release, which in some susceptible individuals may produce a pseudoallergic response.

### *Choice of Narcotic Analgesic*

Morphine must still rank as the drug of choice among these agents, despite the growing list of opioids. A recent survey of physicians around the world, based on the possibility that opium production might be sharply curtailed, confirmed that it might be possible to supplant opiates with synthetics, but that it would be neither easy nor highly desirable (19). Despite the long use of pethidine, it seems clear that the drug is widely misused, both in terms of dose and dosage schedule (20). Lessened dependence from pentazocine has not been as much an advantage in the clinic as was earlier assumed, and its efficacy as compared with morphine or pethidine has been questioned (21). Still, the choice among the drugs might be best based on the personal preference of patients or some differential response to side effects. While morphine might be the drug to be tried first, intolerance to it clearly demands a search for an alternative and pentazocine might be a good second choice (22).

Morphine is weakly active by mouth, unlike codeine. As oral dosage is often preferred for a variety of reasons, codeine is widely used. In the opinion of the experts surveyed, this use of codeine, in addition to its great efficacy as a cough suppressant, made it virtually irreplaceable (23). Oral pentazocine might be the best alternative choice for relief of pain and dextromethorphan the best for cough suppression.

When narcotics must be used for long periods, such as the treatment of pain of malignancy, many clinicians prefer a long-acting orally active drug, such as methadone. It is regrettable that by administrative fiat this use of methadone has been virtually eliminated in the United States. Dextropropoxyphene is one of several examples of drugs that have been highly successful in the marketplace despite the fact that they are extremely weak. This drug has about 1/25 to 1/50 the potency of morphine. At most tolerable doses, the drug must be combined with non-narcotic analgesics to have any appreciable effect (24).

### *Clinical Indications and Use*

Severe acute pain, such as that following acute myocardial infarction, renal or biliary colic, extensive surgical procedures, burns, and other traumas are primary indications for narcotic analgesics. So, too, is chronic pain of moderate to severe degree associated with malignancies. As relief of pain by narcotic analgesics is one of the most predictable therapeutic interventions physicians can make, it is sad that many physicians misuse these drugs. Either unwarranted fears of producing dependence, ignorance of the proper dose and duration of action of these drugs, or failure to attend carefully to the complaints of patients often leads to their underuse, inflicting unnecessary suffering. Pethidine, because of its low potency and short duration of action, seems to be the drug most likely to be underused (20). Doses of narcotic analgesics should also be adjusted for age, for older patients show consistently greater relief from smaller doses than do younger patients (25).

Morphine is the preferred drug for treating the pain of acute myocardial infarction, and is usually given intravenously to assure the most rapid onset. Small doses of 8–10 mg may be slowly injected, followed by additional increments of 3–6 mg as needed to achieve the desired effect. The principal advantage of the cardiovascular effects of morphine in myocardial infarction, compared to pentazocine, is a lowering of cardiac work (26). Nausea and vomiting from opiates would be most harmful in such patients, so that a prior or concomitant dose of 50 mg of cyclizine has been suggested to mitigate this adverse effect (27). Morphine has first place in the treatment of acute pulmonary edema, whether or not it is associated with acute myocardial infarction, for reasons mentioned earlier. As one cannot be certain that some patients with acute pulmonary edema may not also have varying degrees of respiratory insufficiency concomitantly, close monitoring of blood gases is needed to detect any possible deterioration due to the use of morphine.

Postoperative pain is another clear indication for narcotic analgesics. When patients cannot take drugs orally, as is often the case, parenteral preparations are required. Differences in potency may be considerable, hydromorphone being 8–10 times as potent as morphine, but differences in efficacy are negligible (28). Pethidine is shorter-acting than morphine, which could lead to inadvertent undertreatment, and pentazocine seems to be still shorter-acting (29). When an oral drug can be tolerated, pentazocine may be preferred over pethidine and codeine, other narcotics that have a fairly high degree of activity when given by this route (30). Ratios of dose and duration of action of some commonly used narcotics, using morphine as a standard, are shown in Table 1.

When narcotics are needed for relief of pain associated with chronic malignant disease, the sole guide to treatment should be the comfort of the patient. It is often possible to reduce the doses required if one uses a constant dose of a non-narcotic analgesic, such as aspirin, along with the narcotic, an often overlooked procedure.

**Table 1** Dose relationship among narcotics: ratios to morphine

| Narcotic | Dose | Duration |
|---|---|---|
| Morphine | 1.0 | 1.0 |
| Dihydromorphinone | 0.2 | 1.0 |
| Codeine | 12.0[a] | |
| Pethidine | 7.5[a] | 0.7 |
| Propoxyphene | 24.0[a] | |
| Pentazocine | 4.5[a] | 0.7 |

[a] Moderate to high oral/parenteral ratio.

The production of drug dependence is no problem in this case. Some clinicians feel uneasy when the daily doses of morphine increase to more than 100 mg and feel more comfortable by switching to a more potent drug, so that the milligram dose is less. No special advantage is gained.

It must be constantly reemphasized that doses should be tailored to the patients' needs, as determined by response to the first arbitrarily chosen dose. Intervals between treatment should also be determined by the duration of action of the drug in the patient. No orders for narcotics should be routine, but rather should be based on the needs of each patient. Both the patient and the nurse should be allowed considerable discretion in these matters.

Preoperative use of narcotics has been increasingly questioned. In the absence of pain, it is generally agreed now that they are not required. One wants a mildly sedated, nonanxious patient with reduced vagal activity. Thus, it is possible that one can achieve these goals simply with a barbiturate, such as secobarbital or pentobarbital sodium, and a mild dose of atropine or scopolamine. Intravenous diazepam, in dose of 5–10 mg, along with 0.4 mg of atropine, has also proved to be an effective preoperative medication (31). Although it has been customary to give antihistaminic phenothiazines, such as promethazine, along with preoperative medication, both to augment sedation and possibly to prevent postoperative vomiting, it is questionable whether the latter aim is achieved better than it would be with simple sedation. The autonomic side effects of phenothiazines may confound the effects of anesthetics on pulse rate and blood pressure.

Cardiac surgery has provided a new application for morphine, where it is often used as the primary anesthetic agent. Following induction with nitrous oxide-oxygen, morphine is administered intravenously at a rate of 20 mg every 2 min until consciousness is lost or until a maximum dose of 2 mg/kg has been given. When compared with halothane, the latter drug produced greater fall in blood pressure, but serious hypotension was similar between the two. Intraoperative hypertension was more common with morphine. Otherwise the two drugs were comparable (32). So-called neuroleptanalgesia, using a combination of a pethidine-like drug, fentanyl, with a butyrophenone, droperidol, has fallen into disrepute.

## *Unwanted Effects and Contraindications*

Most unwanted effects of narcotic analgesics are extensions of primary pharmacologic actions and might be anticipated. Generally, they are dose-related, at least so far as intensity is concerned. Pentazocine is not completely free of the potential for dependence, but most reported cases have been easily managed (22). Perceptual disturbances were noted in 9 of 132 hospitalized patients treated with pentazocine, a somewhat higher prevalence than would be expected with other narcotics (33). This particular effect of the drug, which is a racemic mixture, has been attributed to the D-isomer (34). Fibrous myopathy has been described in patients who have been placed on long-term intramuscular administration of pentazocine. The sites near the injection areas, either deltoid or quadriceps, are mainly involved. As the onset of this complication may be subtle, patients in whom long-term parenteral treatment is expected should best be started on another drug (35).

Patients with myxedema do not tolerate sedatives or narcotics well; even conventional doses are adequate to produce frank coma. Thus, untreated myxedema would be a contraindication to these drugs. Pulmonary failure, bronchial asthma, and cor pulmonale may all be aggravated by central depressant drugs, almost all of which, including narcotics, are either contraindicated or require scrupulous attention to their use. Once a patient has been placed on a ventilator, narcotic analgesics may facilitate tolerance to the machine. Compromised metabolism by liver damage is also a reason to be cautious in the use of these drugs, which could precipitate hepatic coma.

## NON-NARCOTIC ANALGESICS

### *Chemical Structures*

Major salicylic acid derivatives include acetylsalicylic acid (aspirin), sodium salicylate, and salicylamide (Figure 2). The latter two are relatively weak analgesics compared with aspirin. Aniline derivatives include acetaminophen, a metabolite of both acetanilid and acetophenetidin. The latter two drugs should now be considered obsolete. The only pyrolone derivative worth considering as an analgesic would be antipyrine, which was unfortunately tarred with the same brush as aminopyrine;

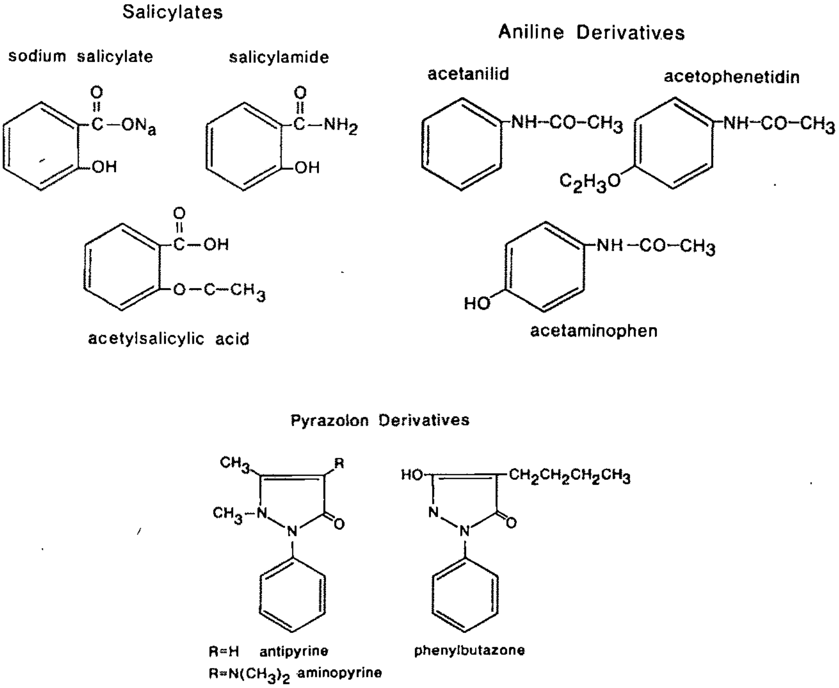

*Figure* 2 Structural relationships between three classes of non-narcotic analgesic drugs.

phenylbutazone is more of an anti-inflammatory than an analgesic drug. The popularity of aspirin is attested to by the more than 200 over-the-counter preparations that contain it (36).

### *Pharmacologic Effects*

Drugs of this class have varying degrees of analgesic, antipyretic, and anti-inflammatory effects (Table 2). Aspirin possesses the best combination of all three effects and remains the standard and unsurpassed drug in the group. Current evidence suggests that the analgesic action of aspirin is peripheral, presumably interfering with various chemical mediators of inflammation and pain, such as bradykinin (37, 38). Recently, aspirin and similar nonsteroidal anti-inflammatory drugs have been found to block prostaglandin synthetase, a property peculiar to this class of drugs. The mechanism of the inhibition of prostaglandin formation is still unclear. Further, effects on synthesis of prostaglandins in different locations may explain some divergence in pharmacological effects. Acetaminophen, which has no anti-inflammatory activity, is extremely weak as a blocker of certain peripheral prostaglandin synthetases, yet it is equal to aspirin in blocking this enzyme in the brain. This observation raises the question as to whether perhaps some analgesic actions of these drugs are mediated centrally as well as peripherally (39).

It is rather remarkable that a drug such as aspirin has such profound effects against the body's defense mechanisms of pain, inflammation, and fever. Some evidence also suggests that it may reduce cell-mediated immunity as well (40). In addition, the effects of blocking platelet aggregation and increasing bleeding time raises the possibility that the drug may be protective against various thrombotic disorders, especially myocardial infarction and venous thrombosis (41, 42).

Aspirin is readily absorbed, with peak levels in plasma attained within 2 hr in most individuals. The elimination rate varies considerably between individuals, the plasma half-life ranging from 2.5–8.5 hr. Prolonged-action dosage forms simply delay the peak of plasma levels and generally reduce total bioavailability. Buffered dosage forms increase the plasma levels of unhydrolyzed aspirin, which may be an advantage (43). Acetaminophen is rapidly absorbed, except in the presence of high-carbohydrate meals containing pectin. It is conjugated in the liver at its *p*-hydroxyl group, predominantly with glucuronic acid and to a minor degree with sulfate. The conjugates are readily excreted in urine. The half-life of the drug is brief, usually 1–2 hr (44).

Table 2 Comparisons among non-narcotic analgesics: degrees of pharmacologic effects[a]

| Analgesic | Analgesia | Antipyretic | Anti-inflammatory |
|---|---|---|---|
| Aspirin | ++ | ++ | ++ |
| Sodium salicylate | ± | + | ++ |
| Acetaminophen | ++ | ++ | + |
| Acetophenetidin | + | + | + |

[a] ++ = strong; + = mild; and ± = weak to absent.

## *Clinical Indications and Uses*

Non-narcotic analgesics are most often used for headache, arthritis, or other pain derived from either the integument or from the musculoskeletal system. Their use in some types of pain usually treated by narcotics, such as pain from burns, wounds, or even chronic malignancy, may have been overlooked. A basic dose of non-narcotic analgesic, while not fully effective alone, might decrease requirements for narcotics.

Aspirin remains the drug of choice among this group, with few exceptions. Using a double-blind crossover technique and single doses, aspirin (650 mg) was superior to all other agents tested. Acetaminophen (650 mg), pentazocine (50 mg), and codeine (65 mg) were also better than placebo and not significantly different from aspirin. Propoxyphene (65 mg) gave no evidence of therapeutic activity and was no different from placebo. Side effects from pentazocine as compared with the others made it of dubious value for ambulatory patients (45). Such results have been repeatedly confirmed, indicating that it is difficult to make a case that any of the commonly used oral analgesics are superior to aspirin, while aspirin is often better than some of the others.

A plateau in response to aspirin may be reached at 1300 mg, which is usually the largest single dose employed. Higher doses simply increase toxicity. The usual dose of aspirin is 650 mg, which does not produce markedly higher peak levels of serum salicylate than doses of 325 mg, but does produce levels that persist longer. Frequency of doses depends on many variables: the rate of elimination by the individual patient, urine pH, and other factors. Thus, it makes little sense to specify an arbitrary time interval between doses but, as with the narcotic drugs, the patient's needs are the best guide. After several doses have been given, the frequency of dose may become less as levels of salicylate and unhydrolyzed aspirin accumulate.

Aspirin is most rapidly absorbed in solution, which may be easily accomplished by adding stoichiometric amounts of sodium bicarbonate. Failing that, buffered preparations may be used, although the gain hardly justifies their increased cost (43). Simplest of all is to take aspirin tablets with 180–240 ml of warm water. Were adequate water taken whenever aspirin is given, the prevalence of gastrointestinal bleeding would doubtless be much reduced.

Acetaminophen is aspirin's only serious competitor, and its use has been increasing steadily. Like aspirin, it is usually found to be more effective than propoxyphene. A dose of 650 mg of acetaminophen was more effective than single doses of 65 mg of propoxyphene (which was in most instances no better than placebo). Adding propoxyphene to acetaminophen did not materially increase pain relief (46). Single doses of 1000 mg of acetaminophen were superior to doses of 65 mg of propoxyphene or placebo in patients with episiotomy pain (47). In this regard, results obtained with acetaminophen are entirely comparable to those from aspirin. Whenever any element of inflammation contributes to pain, acetaminophen might be expected to be inferior to aspirin.

Many new nonsteroidal anti-inflammatory agents have been recently introduced that share some pharmacologic properties with aspirin. Ibuprofen, in doses of 300 and 900 mg, was compared with aspirin in doses of 900 mg in episiotomy pain. All treatments were significantly better than placebo in terms of providing more than 50% pain reduction. Differences between the two doses of ibuprofen were not remarkable (48). While it is conceivable that these drugs might find some use as substitutes for aspirin, acetaminophen would be preferable and cheaper.

Use of aspirin and acetaminophen as antipyretics is about equally effective and both were superior to tepid sponging alone; the latter procedure did not enhance the fever-reducing effects of the drugs (49). However, the proper role of drugs for treating fever is still controversial, and it is easy in one's zeal to treat children with fever to produce an inadvertent overdose (50). Quite possibly, antipyrine might be an equally effective and safer drug than either of the other two for this specific indication.

## *Unwanted Effects and Contraindications*

The dangers of aspirin are real, but may have been somewhat overexaggerated. For instance, occurrence of major gastrointestinal bleeding due to aspirin intake was estimated at 15 per 100,000 chronic users per year, while for uncomplicated benign gastric ulcer, the prevalence rate was 10 per 100,000 per year (51). Thus, it seems reasonable to interdict aspirin in patients with unexplained gastrointestinal bleeding, or in patients with known gastric or duodenal ulcers (52). Others have come to the conclusion that the low incidence of side effects of aspirin is hardly a reason for the wholesale abandonment of the drug (53). The ulcerogenic action of aspirin does not occur when the drug is given in buffered, solubilized form, nor when achlorhydria is present. The mucosal injury from unbuffered aspirin increases cellular permeability to hydrogen ions in the lumen, by a process of back-diffusion (54). As aspirin increases bleeding time, even after usual therapeutic doses and often for several days, this action might exacerbate bleeding from lesions due to other causes. It is also reason for blood donors to exclude aspirin intake for a week prior to giving blood (55).

Analgesic nephropathy remains controversial. Some aver that it may be the result of chronic use of several analgesics, including aspirin, antipyrine, aminopyrine, and phenacetin, and that aspirin is the major toxic component. Phenacetin, and possibly acetaminophen, are believed to play secondary roles (56). Such opinion is at variance with the prevailing view that aspirin by itself is rarely associated with nephropathy, even when taken for long periods of time in high doses (57). Phenacetin seems to be the necessary ingredient for nephropathy, regardless of whether it is the sole toxic agent or acts synergistically with aspirin. As it is a needless agent, the wisest course would be to avoid it altogether or to make it available only on prescription, as has been done in Britain (58).

Aspirin-induced hepatic injury is a newly recognized complication, the exact prevalence and circumstances of which remain to be fully elucidated. It may present as chronic active hepatitis, particularly in patients susceptible to systemic lupus erythematosis (SLE) (59). Dose-dependent hepatocellular damage may be found in patients with no other evidence of SLE, so that aspirin must be considered as a possible cause for any unexplained liver disorder (60).

Aspirin-sensitive asthma tends to occur in middle-aged women with preexisting vasomotor rhinitis and nasal polyps. The overall prevalence of this complication is slight, but it may be quite high in patients with bronchial asthma. Thus, aspirin is often considered to be contraindicated in these patients. Such aspirin-sensitive patients have been found to show decreased peak expiratory flow rates when exposed to several drugs, all of which shared the action of aspirin in blocking prostaglandin synthesis. Thus, such reactions may not be unique to aspirin, but also occur with indomethacin, phenylbutazone, and other nonsteroidal anti-inflammatory drugs (61). The absence of any skin-sensitizing antibodies to N-acetylsalicylic acid in the serum of sensitive individuals suggested that allergy was not the underlying mechanism. Rather, it was proposed that direct histamine liberation was, and that the syndrome was analogous to other drug-induced anaphylactoid reactions (62).

The importance of the observation that shedding of rhinovirus was markedly increased by aspirin-treated individuals remains to be determined. It could have important epidemiological consequences and raise questions about the practice of treating common colds with aspirin (63).

Acetaminophen has become popular because it is not known to cause gastrointestinal bleeding or disturbances in blood clotting. Therefore, it may be substituted for aspirin in situations in which the latter drug is contraindicated. Rare instances of methemoglobinemia, thrombocytopenia, agranulocytosis, hypoglycemia, and fixed dermatitis have occurred. The most ominous complication, which is being reported more frequently with increased use of the drug, is hepatic damage. These are complications of overdoses, usually in excess of 7 g for an adult and 3 g for a child. Often the onset is insidious, with vomiting for a few hours followed by anorexia, nausea, vomiting, and epigastric pain for the first 24 hr. Liver tests become abnormal through the second to fourth day, when jaundice also appears. If the acute centrilobular necrosis progresses, frank and fatal hepatic failure may occur (44).

The hepatic lesion associated with acetaminophen is caused by microsomal enzyme-induced metabolism of the drug to a highly active intermediate metabolite that binds covalently to liver cell macromolecules. Therapeutic doses are not toxic because small amounts of active metabolite are rapidly inactivated by preferential conjugation with hepatic glutathione and excreted as cysteine or mercapturic acid conjugates. Precursors of glutathione, such as cysteine and cysteamine, enter the cell more readily and supply enough glutathione to inactivate the large amounts of toxic metabolite. This reasoning has led to the experimental demonstration that cysteamine can prevent acetaminophen-induced liver damage in animals. The drug has been used successfully in the clinic, given intravenously as an initial dose of 2.0 g followed by three subsequent 400 mg doses at subsequent intervals of 4, 8, and 8 hr, for a total dose of 3.2 g (64).

It is clear that acetaminophen may be a reasonable substitute for aspirin where the latter drug is truly contraindicated, but it should not be considered a universal replacement for aspirin. Poison kits should now contain freeze-dried ampules of cysteamine for use in toxic emergencies associated with acetaminophen, as it seems to be a specific antidote.

## COMBINATIONS OF DRUGS

The time-honored combination of aspirin and codeine is rational and unquestionably effective. It makes use of the two major points of attack on pain, through the periphery and centrally. Usual combinations include 650 mg of aspirin with 65 mg of codeine, 9.76 mg of oxycodone, or 25 mg of pentazocine. Such combinations are about equally effective and equally tolerable. The same amount of aspirin combined with 65 mg of caffeine, 32 mg of pentobarbital sodium, 25 mg of promazine hydrochloride, 75 mg of ethoheptazine citrate, or 100 mg of propoxyphene napsylate produced no better analgesia than aspirin alone (65).

The combination of narcotic drugs with sedatives, such as pentobarbital sodium or diazepam, may be desirable when one wishes to produce sleep. Although relief of pain alone may induce sleep, this result cannot always be counted on. A combination of pentazocine with oxypertine, a sedative drug, produced more pain relief than the former drug alone (66). It is still uncertain whether the use of dextroamphetamine in combination with narcotics increases analgesia or reduces mental impairment; such use would be limited to daytime hours to try to maintain a normal diurnal sleep rhythm.

Tricyclic antidepressants have become immensely popular drugs in pain clinics, whose patients are predominantly those with chronic pain in which a major affective component has been added. They may possibly have a direct action on nerve conduction, especially when used in combination with phenothiazines. Such combinations have been reported to be effective in postherpetic neuralgia (67).

## CONCLUSIONS

Pain is still the most common complaint for which patients seek attention and one of the most common for which drugs are prescribed. Drugs may not always be the preferred treatment for pain, but are often both the most readily effective and the easiest treatment to administer. Narcotic analgesics are used for severe pain, and among these drugs morphine remains preeminent. Reasonable alternatives are pentazocine and pethidine or, where orally active forms are desirable, codeine or pentazocine. The continued widespread use of propoxyphene, despite considerable evidence that it is at best a very weak analgesic, suggests that physicians still need more education about these drugs.

Non-narcotic analgesics are usually employed for mild pain, but may be used as a basic treatment for patients with severe pain as well. Aspirin, which is truly a wonder drug, remains the epitome of this group. When clear contraindications to the use of aspirin exist, acetaminophen is a reasonable substitute. Indiscriminate use of the latter drug may have more hazards than are currently appreciated.

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# REYE'S SYNDROME

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## INTRODUCTION

In 1963, Reye et al (1) described a clinical and pathological entity in 21 Australian children which he called encephalopathy with fatty degeneration of the viscera and which has become recognized as Reye's syndrome. What had originally been regarded as unusual has since become recognized as a relatively common phenomenon.

It has now been 12 years since Reye's original report, and approximately a thousand cases have been reported. Despite this, several crucial questions remain unanswered: What is the etiology? Are there predisposing factors? What are the pathogenic mechanisms? What is the optimal therapy? Interest in Reye's syndrome is great, and theories concerning etiology, pathogenesis, and treatment are plentiful. Definitive answers should be forthcoming in the near future.

## CLINICAL (1–5) AND LABORATORY (1, 2, 6–9) FEATURES

Reye's syndrome occurs as sporadic cases and in clusters in children of all ages (2, 3, 6). The patients are not distinguished by sex, race, family history, or socioeconomic factors (4). Reye's syndrome can be a rapid, fulminating disease which ends fatally. The earlier reported high mortality rates of 85–100% have been replaced by rates of 35–50%. This improvement reflects the effect of new modes of treatment and increased awareness of the disease.

In most cases of Reye's syndrome there is an antecedent viral-type illness. The duration of the prodromal illness may be hours or days before there are further signs and symptoms, and frequently the patient appears to be recovered from the original disorder. The syndrome is ushered in by a period of protracted, usually severe vomiting soon accompanied by evidence of encephalopathy. At times hematemesis occurs. At first there may be mild lethargy and the child may then proceed through a succession of deepening stages of encephalopathy highlighted by seizures, decerebrate activity, dyspnea, hyperventilation, hyperreflexia, dilated pupils that react to light, extensor plantar reflexes, somnolence, coma, hypoventilation, and decorticate activity. The severity of the encephalopathy appears to be an important indicator of prognosis. Thus, clinical staging has been advocated. The duration and progression of the encephalopathy is variable. Some patients have deteriorated to a certain stage and remained there for days, some have recovered within hours, and others have progressed to deep coma and decorticate activity within hours.

The children can exhibit many symptoms: they are usually afebrile, have hepatomegaly which may first appear as the encephalopathy progresses, are not jaundiced, may have signs of metabolic acidosis or respiratory alkalosis, and may have signs of increased intracranial pressure and electrolyte imbalance.

The liver enzymes, serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), are elevated. Other enzymes have been abnormal where measured, including creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). The prothrombin time is usually increased. The blood ammonia level is greatly elevated in most patients in whom it has been assayed. Aminoacidemia with elevation of alanine, lysine, and glutamine; aminoaciduria; low serum levels of citrulline; and free fatty acidemia have been found in some patients. Hypoglycemia, a prominent feature in Reye's original group of patients, is not a consistent finding. Laboratory evidence for metabolic acidosis occurs in 75% of the patients; respiratory alkalosis is also found. Leucocytosis is usually present. The routine cerebral spinal fluid examination is characteristically normal except for increased pressure and low sugar concentration in patients with concomitant hypoglycemia.

## PATHOLOGY

The pathology of Reye's syndrome is characteristic and primarily features changes in the liver and brain and to a lesser extent in the kidneys, heart, and pancreas.

### *The Liver (1, 2, 10–12, 20)*

The gross appearance of the liver in biopsy specimens is yellow-orange, pale yellow, or white. On light microscopy the chief features are fatty degeneration of the cytoplasm of the hepatocytes throughout the lobule, regular arrangement of the cell nuclei near the center of the liver cell plates, absence of inflammation, and virtual absence of cell necrosis. The fat is triglyceride. The main ultrastructural finding is the alteration in the hepatocyte mitochondria. There is swelling, pleomorphism, amoeboid configurations in severe cases, and reduction in the number of mitochondrial dense bodies. The mitochondrial lesion is reversible. There is progressive reduction in glycogen content proportional to the clinical severity, proliferation of endoplasmic reticulum, and increased number of peroxisomes.

### *The Brain (1, 11, 13)*

The pathologic changes found in the brain have essentially been cerebral edema and anoxic neuronal degeneration, but no gross inflammation. The meninges are normal. In most cases the brains were enlarged with or without tonsillar coning or herniation, and marked flattening of the gyri. Cerebral edema appears to be the most marked microscopic alteration. Throughout the cortex there are anoxic neuronal changes. Swollen astrocytes and pleomorphic microglial cells have been observed, as has loss of Purkinje's cells in the cerebellum.

### *Other Organs (1, 11, 14)*

Fatty degeneration in the proximal convoluted tubules and in the loops of Henle of the kidney have been observed. Fatty infiltration of the myocardium has also been described. In the pancreas, fatty degeneration and intranuclear inclusions in the acinar cells have been reported.

## ETIOLOGY AND PATHOGENESIS

Definition of the exact etiology and pathogenesis of Reye's syndrome remains to be determined. The association with viruses is a strong one, yet at the same time the pathological processes resemble the effects of toxins. Viruses may act merely as triggering mechanisms for exogenous or endogenous toxins, may exert a direct toxic effect, or may interact with toxins in a synergistic fashion.

The syndrome has been associated with multiple viruses. The most frequent virus involved seems to be influenza B, and clusters of Reye's syndrome patients have appeared in relation to outbreaks of influenza B (3, 15). Earlier, Norman et al (16) had successfully isolated influenza B from the liver of a Reye's syndrome patient. Varicella virus has been implicated in about 10% of children with Reye's syndrome. Other viruses that have been isolated from cases of Reye's syndrome (2, 3, 12, 17) include reovirus 1 and 2, ECHO viruses 2, 8, and 11, Coxsackieviruses A, $B_4$, and $B_5$, adenoviruses, herpes simplex, influenza $A_2$, parainfluenza, cytomegalovirus, rhinoviruses, and the Epstein-Barr virus of infectious mononucleosis. Clinically, patients have had antecedent or concomitant rubeola, herpes zoster, and vaccina (2). In some instances there was evidence of mixed viral infections (3, 12).

Exogenous toxins have been intimately associated with Reye's syndrome in certain patients. Becroft first suggested that aflatoxin might be implicated (18). Recently, the disease has been reported to be endemic in certain areas of Thailand during the rainy season when contamination of foodstuff with aflatoxin is high (19, 20). The affected Thai children had similar clinical, biochemical, and histologic findings to other cases of Reye's syndrome. Aflatoxins B1 and B2 were found at autopsy in the liver and the gastrointestinal tract. Administration of aflatoxin to young monkeys produced a similar pathologic process (21). Hypoglycin, a toxin found in the unripe fruit of the ackee tree, has been found to produce Jamaican vomiting sickness, a disease that closely resembles Reye's syndrome clinically and pathologically (22). However, many epidemiological investigations and searches for potential toxins both in patients and in their environment have been negative in regard to implication of a toxin. Aspirin has been suspected as playing a prominent role in the disease dynamics, as most patients receive some form of aspirin during their prodromal period. In most patients the salicylate levels have been normal (3).

Theories concerning the pathophysiological mechanisms in Reye's disease are numerous. Glasgow et al (23) showed that the hypoglycemia of Reye's syndrome was secondary to decreased hepatic glucose production. Evans et al (13) proposed that the liver receives the primary injury, which alters metabolic processes, causing hypoglycemia and fatty metamorphosis of the viscera. The hypoglycemia, plus an accompanying acidosis and possibly the etiologic agent(s), causes anoxic neuronal degeneration and cerebral edema, which in turn are responsible for the major signs and symptoms and death. Brown & Madge (24) presented evidence that the systemic and metabolic disturbances in Reye's syndrome were primarily a result of excessive fatty acid mobilization with the fatty acids acting as endogenous toxins. The fatty acids are capable of producing an encephalopathy and altering mitochondrial metabolism and structure. Schubert et al (2) presented the following chain of events: (*a*) vomiting and starvation due to an infection cause tissue fatty acid mobilization; (*b*) an endogenous or exogenous toxin prevents normal mitochondrial metabolism of the mobilized free fatty acids; and (*c*) the cytoplasmic free fatty acids cannot be removed by the mitochondria and are converted to triglycerides or bound to cytoplasmic binding sites. The mitochondrial injury would be reversible up to a point. Huttenlocher et al (7) stressed hyperammonemia as the critical factor in the encephalopathy of Reye's syndrome. Several investigators have demonstrated disturbances in the urea-cycle pathways of Reye's syndrome patients, which permit ammonia to accumulate in the blood (8, 9, 25). They found high concentrations of glutamate, alanine, and lysine, and absence or very low concentrations of citrulline, due to a deficiency of the mitochondrial enzyme ornithine transcarbamoylase. Some of the above findings and theories account for various features of Reye's syndrome. The pieces of the puzzle are falling in place but the ultimate solution might depend on the development of a suitable animal model.

## TREATMENT

The treatment of Reye's syndrome has varied through the years. As a better understanding of the operative pathophysiology has progressed, different modes of treatment have been applied.

Supportive measures are important. These consist of programs to correct electrolyte imbalance, metabolic acidosis, and respiratory alkalosis; and respiratory assistance when needed. Prompt treatment of increased intracranial pressure may be lifesaving. Kindt et al (26) recommend close monitoring of intracranial pressure and use of dexamethasone, regulation of arterial blood gases, and cerebral dehydrating agents (mannitol and/or urea). Craniotomy for decompression should also be considered. Reye (1) pointed out the necessity to correct the hypoglycemia with intravenous glucose. Brown & Madge (27) have advocated the administration of glucose plus insulin to reduce lipolysis and mobilization of fats with production of fatty acids. Huttenlocher et al (7), stressing the pathogenic role of the hyperammonemia, recommended measures similar to that used in hepatic coma: low protein intake, cleansing enemas, intestinal tract sterilization. The recent reports on derangement of the urea cycles with resultant hyperammonemia and ammonacidemia and low concentration of citrulline caused by ornithine transcarbamoylase deficiency have resulted in recommendations to administer ornithine or arginine, preferably by mouth, or to use citrulline (9, 28). The latter has been used to treat three patients successfully (9).

Two other modes of treatment have been advocated: peritoneal dialysis and exchange transfusions. Early reports of treatment with peritoneal dialysis were quite favorable in 24 cases of Reye's syndrome (29), but later reports included a high failure rate (30). Exchange transfusions have been used in Reye's syndrome patients (2, 31). In one series, 5 of 11 patients with severe encephalopathy were exchanged and recovered, while all 11 in the same clinical category who were not exchanged died (2).

It is essential that the different modes of treatment receive critical evaluation. Proper control studies are needed and would have to be conducted on a collaborative basis. Most important in the evaluation would be uniform staging of the degree of encephalopathy at the time of initiation of therapy.

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# HYPERSENSITIVITY PNEUMONITIS: CURRENT CONCEPTS OF ETIOLOGY AND PATHOGENESIS

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## INTRODUCTION

Hypersensitivity pneumonitis or extrinsic allergic alveolitis can be defined as a group of diffuse interstitial and/or alveolar-filling "granulomatous" pulmonary diseases, associated with intense respiratory exposure to finely dispersed organic dusts of appropriate particle size. These diseases are characterized by symptoms and signs attributable to a reaction in the peripheral part of the bronchial system giving rise to defects in gas exchange (1). Clinically, affected patients have episodes of fever, cough, and dyspnea 4 to 6 hours following exposure to an organic dust (hay, bagasse, pigeon droppings, etc). These acute symptoms are frequently mistaken for those of bacterial or viral pneumonia. In more insidious cases associated with long-term exposure to small quantities of antigen, an afebrile, chronic form of the disease may occur, often associated with dyspnea, malaise, weakness, and weight loss. Functional abnormalities in classic cases consist of defects in alveolar gas exchange, arterial oxygen unsaturation, and decreased pulmonary compliance. Mild but persistent airway obstruction has been reported, and changes similar to those found in emphysema, including irreversible obstructive and restrictive defects with severe disturbances in diffusion capacity may be seen in some chronic cases.

The basic pathology in these diseases consists of inflammation of the alveoli and interstitial pulmonary tissue, plus some involvement of bronchioles. In acute stages, there is thickening of alveolar septi with predominant lymphocyte and plasma cell infiltrates. In many cases collections of foamy macrophages are noted within the alveoli. In more chronic stages, noncaseating granulomas with epitheloid cells, Langerhans' giant cells, and various degrees of fibrosis are observed. Pulmonary vasculitis as noted in Arthus-type reactions (type III hypersensitivity) is almost always absent, although there have been occasional reports of vasculitis in acute cases (2, 3).

Although pulmonary disease associated with organic dust inhalation has been known since 1713 (4), the first modern report appeared in 1932 when farmer's lung was described as an acute pneumonitis occurring in workers exposed to moldy hay (5). In subsequent years an expanding list of similar diseases associated with a variety of occupations and avocations has been described. The entire subject has been thoroughly reviewed by Pepys (1) and others (6–8). It is the intent of this paper to review some of the recent advances in etiology and pathogenesis of hypersensitivity pneumonitis obtained by using animal models.

## ETIOLOGY

There are numerous causes of hypersensitivity pneumonitis and the list continues to expand as awareness of this possibility increases and newer forms of industrial and agricultural exposure develop. A list of causes is summarized in Table 1. Among the most important etiological agents are thermophilic and mesophilic actinomycetes including *Micropolyspora faeni, Thermoactinomyces vulgaris, Thermoactinomyces sacchari,* and *Thermoactinomyces candidus* (9–13). Actinomycetes are members of the true bacteria (Eubactereriales), although they have the morphology of fungi. These organisms grow best in decaying organic matter such as hay and bagasse, under optimal conditions of humidity and high temperature ranging up to 60°C. The influence of these conditions is shown clearly in hay; moldy hay with approximately 40% moisture content and temperature between 40 and 60°C will support abundant growth of actinomycetes in contrast to poor growth in fresh hay. In addition to composts, actinomycetes are abundant in soils, foods, fresh waters, and other natural sources. They have also been isolated from the atmosphere.

A wide range of antigens has been identified in several actinomycetes by immunoelectrophoresis (IEP) and chemical analysis. At least 29 antigens have been described in *M. faeni* by IEP (14) and the properties and composition of actinomycete antigens are currently under active investigation in several laboratories. The antigens include "metabolic" and "cellular" polysaccharides and glycoproteins with various enzymatic activities (15–18). At present the relative clinical importance of these antigens has not been established. Edwards (19) has demonstrated the presence in *M. faeni* of extremely thermolabile antigens not present in moldy hay. Patients with farmer's lung have antibodies to these antigens, suggesting that at least part of the antigenic stimulus is produced by minimal growth and development of spores in the lungs of these patients. Fungal spores and hyphal fragments may also serve as important sources of antigen in other forms of hypersensitivity pneumonitis. For example, *Aspergillus clavatus* has been shown to produce disease in malt workers and *Alternaria sp.* are associated with wood dust pneumonitis. In pigeon breeder's lung, on the other hand, pigeon droppings are a rich source of antigen. Extensive studies have shown that undegradable pigeon serum proteins with the mobility of gamma globulins are important antigens in this disorder (20–22). Many other antigens in organic dusts associated with these diseases have not been adequately characterized and better understanding of the mechanisms by which these materials cause clinical disease is necessary. The antigens studied have been analyzed within the framework of their capacity to produce precipitating antibodies in humans or in experimental animals. This approach narrows the studies to a limited area of the immune response. It is also likely that some of these inhaled organic dusts

**Table 1** Causes of hypersensitivity pneumonitis

| Disease | Source of antigen | Probable antigens |
|---|---|---|
| Vegetable products | | |
| Farmer's lung | moldy hay | thermophilic actinomycetes<br>*Micropolyspora faeni*<br>*Thermoactinomyces vulgaris* |
| Bagassosis | moldy pressed sugarcane (bagasse) | thermophilic actinomycetes<br>*Thermoactinomyces sacchari* |
| Mushroom worker's disease | moldy compost | thermophilic actinomycetes<br>*Micropolyspora faeni*<br>*Thermoactinomyces vulgaris* |
| Suberosis | moldy cork | unknown |
| Malt worker's lung | contaminated barley | fungi<br>*Aspergillus clavatus* |
| Maple bark disease | contaminated maple logs | fungi<br>*Cryptostroma corticale* |
| Sequoiosis | contaminated wood dust | fungi<br>*Graphium* sp.<br>*Pullularia* sp.<br>other fungi |
| Wood pulp worker's disease | contaminated wood pulp | fungi<br>*Alternaria* sp. |
| Humidifier lung | contaminated home humidifier and air conditioning ducts | thermophilic actinomycetes<br>*Thermoactinomyces vulgaris*<br>*Thermoactinomyces candidus* |
| Paprika slicer's lung | moldy paprika pods | fungi<br>*Mucor stolonifer* |
| Grain measurer's lung | cereal grains | unknown |
| Thatched roof disease | dried grass and leaves | unknown |
| Tobacco grower's disease | tobacco plants | unknown |
| Tea grower's disease | tea plants | unknown |
| Coffee worker's lung | green coffee bean | unknown |
| Hypersensitivity pneumonitis | sawdust | unknown |
| Coptic disease | cloth wrappings of mummies | unknown |
| Animal products | | |
| Pigeon breeder's disease | pigeon droppings | pigeon serum protein (albumin, gamma globulin, and others) |
| Duck fever | bird feathers | chicken proteins |
| Turkey handler's disease | turkey products | turkey proteins |
| Insect products | | |
| Miller's lung | wheat weavils | *Sitophilus granarius* |
| Bacterial or viral products | | |
| Hypersensitivity pneumonitis | *B. subtilis* enzymes[a] | *Bacillus subtilis* |
| Smallpox handler's lung | smallpox scabs | unknown |

[a] Probably induces IgE mediated obstructive airways disease in the great majority of cases.

and microorganisms serve as immunologic adjuvants or act as irritants (23). Evidence indicates that there are varying amounts of antigenically inert (as measured by antibody response) macromolecules present in *M. faeni* extracts (24). Such materials could conceivably be very important in activation of macrophages or of the alternate complement pathway. It seems clear that definition and characterization of antigens is essential for the development of more precise diagnostic tests. More data are also needed on identification of antigens for purposes of skin testing, serum antibody detection, and use in in vitro studies of delayed hypersensitivity in these diseases.

## PATHOGENESIS

The manner by which inhaled organic dusts induce lesions of hypersensitivity pneumonitis is likely dependent on a complex interrelationship between environmental, genetic, and other host-related factors. With regard to organic dusts, several factors may play an important role in their ability to induce pulmonary disease. Very heavy exposure to the offending dust is usually necessary to produce symptoms and it has been calculated that a farmer working with moldy hay can inhale and retain in his lungs 750,000 spores per minute, 98% of which are actinomycete in origin ($<1.0\mu$ in diameter) (25). Although the majority of hypersensitivity pneumonitis cases have been associated with inhalation of dusts containing bacteria and fungi capable of growth and reproduction in the host, the bulk of evidence points toward a hypersensitivity reaction rather than an infectious process. These organic dusts may enhance the immune response in many ways, including a possible adjuvant effect and an effect on macrophage activation. Toxic factors might also contribute to the induction of pulmonary lesions since potent enzymes have been detected in extracts of pigeon droppings (26) and thermophilic actinomycetes (16).

With regard to the host response, one of the more important determining factors might be the association of HL-A haplotypes with disease. In one recent study of pigeon breeder's disease, HL-A 1, 8 was frequently found in association with active disease, suggesting the presence of an HL-associated immune response (Ir) gene in hypersensitivity pneumonitis (27).

There is little evidence for participation of IgE mediated reactions (Type I) in these diseases; patients with hypersensitivity pneumonitis have normal IgE levels (28), eosinophilia is usually not present, and the disease incidence is not increased in atopic patients. Immune complex induced allergic tissue injury (Type III) has been thought to play an important role in the pathogenesis of hypersensitivity pneumonitis. The time interval between exposure and symptoms; the presence of precipitating antibodies in most patients against the offending organic dust; and the detection of antigen, immunoglobulins, and complement components in lung lesions are among the arguments favoring this hypothesis. However, precipitating antibodies to organic dusts can be detected in a large percentage of unaffected exposed subjects (29) and lesions characterized by pulmonary vasculitis, as might be expected in immune complex reactions, usually are not seen in the disease, providing evidence against this hypothesis. Although complement fixing antibodies have been detected in affected patients, the role of complement in disease production is unknown. During provocative challenge of asymptomatic and symptomatic pigeon breeders, complement levels were seen to decline in the asymptomatic rather than the symptomatic patients (30). In patients with pigeon breeder's disease, data have been obtained (31) that suggest the presence of an unusually labile variant of $C_3$ proactivator, which when activated by components of pigeon droppings, initiates activation of $C_3$. Other investigators (32) have suggested that the presence of complement components on the surface of alveolar macrophages of patients without evidence of necrotizing vasculitis is more consistent with the hypothesis that cytotoxic (Type II) allergic tissue injury plays an important role in disease pathogenesis. Recent evidence also indicates that organic dust antigens can directly activate the alternate complement pathway, implying an ability to produce disease on a nonimmunologic basis (33).

There is increasing evidence for a role of delayed (Type IV) hypersensitivity in disease pathogenesis and the pathologic changes in these diseases are consistent with those of delayed hypersensitivity. Antigen induced migration inhibition factor (MIF) production plus lymphocyte transformation, both of which are thought by many to be in vitro correlates of delayed hypersensitivity under certain circumstances, have been detected in these patients and these tests seem to correlate with disease activity (30, 34, 35). Skin testing with protein antigens such as pigeon serum has, however, generally resulted in dual (Type I and III) rather than delayed (Type IV) responses in man, but it is possible that the presence of immediate reactions might obscure the expression of subsequent delayed hypersensitivity reactions. Furthermore, in animal models of these diseases where thermophilic actinomycete antigens have been employed for respiratory tract immunization, classic delayed hypersensitivity reactions at 24 and 48 hr have been produced (36). Based on the currently available evidence it is perhaps naive to assume that only one immunopathogenic mechanism is involved in disease production. It is likely that in the final analysis several types of allergic induced tissue injury as well as nonimmunologic factors may be operative in the pathogenesis of hypersensitivity pneumonitis.

## ANIMAL MODELS

In an attempt to gain insight into the pathogenesis of hypersensitivity pneumonitis, animal models have recently been developed. The different animal species, antigens, routes of immunization, and methodology employed make analysis of these models and their specific applicability to the human disease difficult to assess. A complete review of these models is beyond the scope of this manuscript; rather, we discuss primarily our own experiences plus those of selected investigators as illustrative examples.

With regard to acute pulmonary immunologic injury several models have been developed in the rat and the rabbit. In one such model, intrabronchial inoculation of anti-bovine serum albumin (BSA), followed by administration of labeled antigen intravenously, resulted in acute hemorrhagic neutrophil-rich exudates appearing within the pulmonary parenchyma within 4 hr. By immunofluorescence, interstitial deposits of antigen and antibody, and accumulation of radiolabeled $C_3$ in developing lesions were noted in this model (37). In a similar rabbit model (38) animals were immunized so as to produce a hyperactive response to intravenously administered BSA. When maintained in a state of relative antigen-antibody equivalence by administration of multiple daily doses of BSA, these animals developed proliferative and/or membranous pulmonary lesions associated with deposits of host immunoglobulins, antigen, complement, and fibrinogen. These two models of immune complex induced allergic tissue injury may bear a resemblance to lesions seen in certain early and late forms of human interstitial pneumonitis. Although elegant in design, they do not seem to correspond to human hypersensitivity pneumonitis lesions, which are characterized by mononuclear infiltrates with epitheloid and Langerhans' giant cell formation in the later stages.

Other recent animal models have focused specifically on the production of pulmonary lesions that closely resemble those seen in human hypersensitivity pneumonitis by employing organic dust and actinomycete antigens. Some of these models, which involve active immunization of rabbits, guinea pigs, mice, and rats, follow. In an early model of pigeon breeder's disease, Fink and co-workers exposed rats to daily long-term inhalation of pigeon dropping extract and were able to produce a serum precipitating antibody response plus pulmonary lesions characterized by mononuclear cell infiltrates (39). In our laboratory similar models employing rabbits immunized intratracheally (i.t.) with *Micropolyspora faeni,* a rich source of farmer's lung hay antigen, were developed (36, 40). This type of i.t. immunization resulted in serum precipitating antibodies, delayed skin reactivity, antigen induced peripheral blood lymphocyte transformation, and alveolar macrophage migration inhibition. The immunologic events were associated with intense alveolar and interstitial mononuclear cell pulmonary infiltrates. These early findings indicated that in the rabbit and rat, intense local respiratory tract immunization could initiate a systemic immune response and could produce interstitial pneumonitis with pathologic characteristics similar to those noted in the human disease. Several excellent animal models, which greatly enhanced our understanding of the immunopathogenesis of hypersensitivity pneumonitis, were also developed in the rabbit and guinea pig at this time by Richerson and co-workers (41). In the guinea pig these investigators employed both antigens, which favored the development of delayed hypersensitivity such as tuberculin and azobenzene arsenate N-acetyltyrosine, plus antigens such as ovalbumin, which favored antibody formation. They noted that animals immunized with the former antigens in Freund's complete adjuvant, when challenged by aerosol, developed mononuclear cell infiltrates consistent with the time course of delayed hypersensitivity. On the other hand, when ovalbumin was employed as antigen, acute hemorrhagic pneumonitis lesions rich in neutrophils occurred following aerosol challenge. These animals also developed positive gamma-1 globulin, passive cutaneous anaphylaxis (PCA) responses. In other experiments these investigators administered ovalbumin in Freund's complete adjuvant followed by aerosol challenge and noted development of a mononuclear cell type alveolitis at 24 and 48 hr. Miyamoto & Kabe (42) developed a similar model employing guinea pigs immunized with Freund's adjuvant and tuberculin followed by tuberculin aerosol challenge. In this model striking mononuclear cell infiltrates were also produced 24–48 hr after aerosol challenge and these lesions could be passively transferred with sensitized lymphoid cells.

In an attempt to demonstrate a role for either circulating antibody or delayed hypersensitivity in the production of experimental pneumonitis, hyperimmune serum and specifically sensitized lymphoid cells from animals previously immunized with ovalbumin were transferred to normal rabbits followed by ovalbumin aerosol or intratracheal challenge in our laboratory (43). Rabbits passively sensitized with hyperimmune serum demonstrated only mild and inconsistent peribronchial mononuclear cell infiltrates following aerosol challenge. Animals given between $2.5 \times 10^8$ and $10.9 \times 10^8$ sensitized lymphoid cells intraperitoneally followed by aerosol challenge developed markedly hyperplastic peribronchial lymphoid tissue with germinal center formation and peribronchial mononuclear cell infiltrates following challenge via the respiratory route. The cellular composition of these peribronchial and alveolar lesions in sensitized challenged recipients was consistent with that noted in delayed hypersensitivity reactions. Large numbers of mature plasma cells were also noted in the lesions, suggesting either an additional process of active antibody synthesis by antigen-specific B cells passively transferred along with T cells and macrophages, or recruitment of host B lymphocytes by lymphokines. Attempts at passive cell transfer in this species by the i.v. route have been unsuccessful due to the development of pulmonary vasculitis (41). In other studies of this type, passive transfer of hyperimmune serum or sensitized cells separately in the monkey followed by challenge with pigeon dropping extract (PDE) has been associated with either mild exudative pneumonitis, alveolar hemorrhage, or peribronchial edema (44). These studies suggested that both antibody and sensitized lymphoid cells may play a role in production of pulmonary lesions. Similar results have also been obtained in the guinea pig by passive transfer of serum and cells (45).

In view of the fact that human hypersensitivity pneumonitis is associated with granuloma formation, several models have recently been developed in an attempt to provide information on mechanisms involved in pulmonary granuloma production. Among these are studies by Leake & Myrvik (46) and Moore, Myrvik & Leake (47) in which intravenous inoculation of Freund's adjuvant or BCG has led to the induction of "chronic" and "accelerated" pulmonary parenchymal granulomas associated with MIF production by alveolar wash cells but no dermal delayed hypersensitivity. These pulmonary granulomas have been shown to be antigen specific. They are also inhibited in vivo by cortisone acetate and antimacrophage serum (48). Other workers have employed intravenously inoculated bentonite particles or polyacrylamide beads coated with soluble proteins (hemocyanin, mycobacteria, histoplasma, and schistosomal antigens) (49, 50). In these models, localized multifocal pulmonary granulomas have been produced following i.v. inoculation of antigen coated particles. The granulomas are noted to increase markedly in size and become "accelerated" or augmented when antigen coated particles are administered to previously sensitized animals. Some of these lesions have the histologic appearance of delayed hypersensitivity reactions and can be transferred with specifically sensitized lymphoid cells (51).

In more recent studies in the mouse and chicken by Boros & Warren employing schistosome eggs (51), it has been clearly demonstrated that hypersensitivity pulmonary granulomas are an expression of delayed (cell mediated) hypersensitivity and can be correlated with other in vivo and in vitro parameters of delayed hypersensitivity. In these studies concerned with modulation of schistosomal granulomatous hypersensitivity in the lung, the pulmonary granulomas were shown to be antigen specific and to possess the cellular composition of delayed hypersensitivity reactions. They were inhibited by neonatal thymectomy and could be induced in recipients following passive transfer with sensitized lymphoid cells. Furthermore, their presence did not correlate with antibody levels. Willoughby and co-workers (52) have added to these observations by recently developing an animal model employing nonspecific T-cell mitogens such as concanavalin A, with or without soluble protein antigen administered via the respiratory route. T-cell mitogens alone produced focal interstitial inflammation of the lung, with cells predominantly mononuclear in nature, many blast forms, and some giant cells. In animals sensitized to a soluble protein antigen (BSA) followed by aerosol challenge with antigen and mitogen, vasculitis with interstitial inflammation and necrosis plus severe granulomatous injury bearing a resemblance to Wegener's granulomatosis were noted. Results of this study suggested that repetitive exposure to T-cell mitogens induced interstitial pulmonary lesions and that specific antigen challenge at a time when T-cell mediated injury was also in progress provoked an enhanced type of pulmonary injury. The authors concluded that T-cell stimulation might be quite important as an initiating event in immunologically mediated pulmonary tissue injury resulting from inhalation exposure.

In our laboratory we have recently begun further investigation on the role of delayed hypersensitivity and the alveolar macrophage in experimental granulomatous pneumonitis. In these studies, repeated respiratory tract exposure of rabbits to *Micropolyspora faeni* antigen has led to an early necrotizing inflammatory response of the respiratory bronchioles followed by gradual organization and interstitialization of peribronchial granulomas (43, 53). In some cases progressive bronchiolar fibrosis with destructive lesions similar to those noted in human centriacinar emphysema developed by one month after initial inoculation. Granulomas often assumed the appearance of hard tubercles, including Langerhans' giant cells, which at times contained asteroid and Schaumann bodies similar to those noted in sarcoidosis and human hypersensitivity pneumonitis. We have been unable to detect necrotizing vasculitis or vascular deposits of complement components and immunoglobulins in these lesions by fluorescent microscopy, although $C_3$ was demonstrable in alveolar macrophages and some plasma cells staining for IgG, A, and M were scattered in and about the developing lesions. Alveolar wash cell counts were significantly elevated following i.t. immunization with actinomycete antigen when compared to saline inoculated controls. Glucose oxidation by alveolar wash cells of these animals was also significantly increased over that of saline inoculated controls and morphologic evidence of macrophage activation was noted on electron microscopy. However, phagocytosis of *Staphylococcus aureus* by alveolar macrophages of *M. faeni* inoculated animals was not significantly increased above that of controls, nor were acid protease or lysozyme levels elevated, suggesting that perhaps the total number of activated macrophages was relatively small.

Concurrent with granuloma formation in this model, antigen induced alveolar macrophage migration became progressively inhibited by both direct and indirect assay, and animals developed specific precipitating antibody and delayed skin reactivity. Anti-*M. faeni* precipitating antibody was also exhibited in concentrated bronchial wash fluids of sensitized animals. The time course and histologic features of pulmonary granulomatous lesions suggested that a combination of direct irritation and subsequent hypersensitivity were operative in disease pathogenesis. Overall, these results suggest that multiple immunologic and nonimmunologic processes develop simultaneously at the bronchopulmonary level following respiratory tract exposure to thermophilic actinomycete antigen and it is reasonable to postulate that such a heterogeneous response also occurs in the human disease.

In conclusion, it would appear that a variety of soluble and particulate antigens may induce acute or chronic pulmonary infiltrates in several animal species. These lesions may be associated with Type IV (cellular), Type III (immune complex), and possibly Type II (cytotoxic) allergic tissue injury, depending on the experimental conditions employed. It is also likely that particulate organic dust antigens may possess adjuvant, irritant, alternate complement pathway activating, or toxic properties, all of which could be important in the production of pulmonary lesions.

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# THE SLEEP APNEA SYNDROMES[1] ❖7215

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## INTRODUCTION

For many years pneumologists have conducted extensive studies of alveolar hypoventilation syndromes whose hallmarks are a combination of hypercapnia and alveolar hypoxemia. Nonspecific alveolar hypoventilation can occur secondary to damaged respiratory centers in the brain, as seen in bulbar poliomyelitis (1), brain stem infarct (2, 3), bilateral cervical cordotomy (4), or the uncommon "primary" alveolar hypoventilation syndrome (Ondine's Curse syndrome), first reported by Severinghaus & Mitchell (5). A nonspecific alveolar hypoventilation can also be seen with drug intoxication (barbiturates and tranquilizers) (6) and with abnormalities of breathing apparatus, as in muscular dystrophy, kyphoscoliosis, Pierre Robin syndrome, obstructive lung disease, etc (7–10).

During the 1950s another alveolar hypoventilation syndrome received much attention from respiratory specialists. This was the Pickwickian syndrome, a term coined by Sir William Osler (11) and classically described by Burwell et al (12). In its classic form the Pickwickian syndrome includes obesity, hypersomnolence, periodic breathing with hypoventilation, and cor pulmonale. Several variants have also been described, depending chiefly on the presence or absence of cardiovascular problems and alveolar hypoventilation during wakefulness. For example, Alexander

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and his colleagues (13, 14) reported a "Joe" type of Pickwickian syndrome characterized only by obesity and hypersomnolence. However, in contrast to the alveolar hypoventilation syndromes mentioned above, it was difficult to specify a common underlying physiopathology that would account for all the clinical symptoms and associations seen in the various "Pickwickian" syndromes.

In 1965, Gastaut and his colleagues began to approach the Pickwickian syndrome with a different interest—sleep—and in so doing, they recorded multiple respiratory pauses during sleep (later to be called sleep-induced apnea) in Pickwickian patients (15). Their report inaugurated a new dimension in understanding the complex problems of the Pickwickian syndromes. Clinical sleep specialists began to investigate respiratory changes as a function of sleep. This led to a greater emphasis on the daytime hypersomnolence, and patients began to be seen for sleep disorders rather than cardiorespiratory problems. This difference in patient recruitment gave new data, not previously available to pneumologists, on important interactions between respiration and sleep. Hallmark observations on sleep-induced respiratory dysrhythmias in nonobese patients were made by Duron et al (16), Lugaresi et al (17), and Kuhlo et al (18).

At the suggestion of a respiratory specialist (P. Sadoul) and a neurologist-sleep researcher (E. Lugaresi), the first symposium on sleep-related respiratory problems that included specialists from both areas was held in Italy in 1972 (19). As a result of this intellectual confrontation, a new concept of "sleep-induced apnea syndromes" with secondary cardiovascular consequences has rapidly evolved. In spite of crippling hypersomnolence or hemodynamic problems, the primary respiratory abnormality is typically completely undetectable when patients are fully alert, and obesity is not at all a necessary feature. This group of closely related syndromes has implications for neurologists, cardiologists, otolaryngologists, pneumologists, pediatricians, and, particularly, physicians with a special interest in sleep disorders. Because many reports involving these syndromes have been published in the European and Japanese literature, the primary goal of this review is to facilitate a greater familiarity with sleep apnea syndromes among American physicians.

It is obvious that medical centers lacking a mechanism to ensure adequate laboratory evaluation of the sleep patient who presents a serious sleep disorder will not identify or diagnose a sleep apnea syndrome, since a reliable diagnosis is heavily dependent upon standard sleep recordings (20) combined with respiratory measurements. Such a mechanism has been available at Stanford University for more than five years in the form of a sleep disorders clinic to which such patients can be referred for specialty evaluation. As a result, a uniquely large case series of sleep apnea patients has been evaluated, and a large reservoir of clinical experience with such patients has been accumulated. Because of this, and because most published reports have focused on limited areas of the problem, much of this review is based upon the Stanford University Sleep Disorders Clinic case series, particularly the clinical symptomatology, and unless otherwise specified, the reader may assume that this series is the source of specific descriptive material. Various subsamples of this case series have been described elsewhere (21–24).

## TERMINOLOGY AND DEFINITIONS

An *apnea* has been defined as a cessation of airflow at the nose and mouth lasting at least 10 sec (21); shorter respiratory pauses are discounted. A *sleep apnea syndrome* is diagnosed if, during seven hours of nocturnal sleep, at least 30 apneic episodes are observed both in rapid eye movement (REM) and non–rapid eye movement (NREM) sleep, some of which must appear in a repetitive sequence in NREM sleep. Apneic episodes at sleep onset or accompanying bursts of rapid eye movements in REM periods (25) are not considered pathologic. The foregoing criteria are based on more than 150 all-night polygraphic recordings in a control population of normal adult and child volunteers. Diagnosis is rarely a problem, however, since most patients have hundreds of repetitive apneas in a single night.

Gastaut et al (26) have distinguished three types of sleep apnea with strain gauge and thermistor recording techniques. *Central apnea* is defined by cessation of airflow past nasal and buccal thermistors, accompanied by cessation of respiratory movements measured from abdominal and thoracic strain gauges. In *upper airway apnea,* airflow past nasal and buccal thermistors is absent in spite of persistent respiratory effort recorded by thoracic and abdominal strain gauges. *Mixed apnea* is defined by cessation of airflow and an absence of respiratory effort early in the episode, followed by resumption of unsuccessful respiratory effort in the latter part of the episode. Duron (27) has raised the possibility that mixed apnea can occur as an upper airway apnea, giving way to a central apnea. We have never observed this sequence. These terms and polygraphic definitions are used by nearly all investigators involved in the sleep apnea syndromes. Figures 1 and 2 illustrate the different types.

## COMMON CLINICAL SYMPTOMS AND OTHER FINDINGS IN THE SLEEP APNEA SYNDROMES

At the Stanford University Sleep Disorders Clinic, between June 1972 and June 1975, we identified sleep apnea syndromes in sixty-two patients using the criteria outlined above. They were diagnosed from approximately 350 patients referred for evaluation of serious sleep problems. The patients included 50 adults; 38 male and 12 female ranging in age from 22–70 years; and 12 children, 11 male and 1 female, 3–14 years old. The patients came from all walks of life and socioeconomic backgrounds. Most were Caucasian, and 10% were black. All patients underwent the same initial diagnostic polysomnographic evaluation, which necessarily included continuous monitoring of EEG, eye movements, muscle tone, airflow, ECG, and respiratory effort during sleep. Additional studies were done on subsamples, depending on the specific questions we wished to assess and the willingness of the patients to participate.

The patients were referred to the Sleep Disorders Clinic primarily for a sleep problem, chiefly daytime hypersomnolence and, less frequently, chronic insomnia.

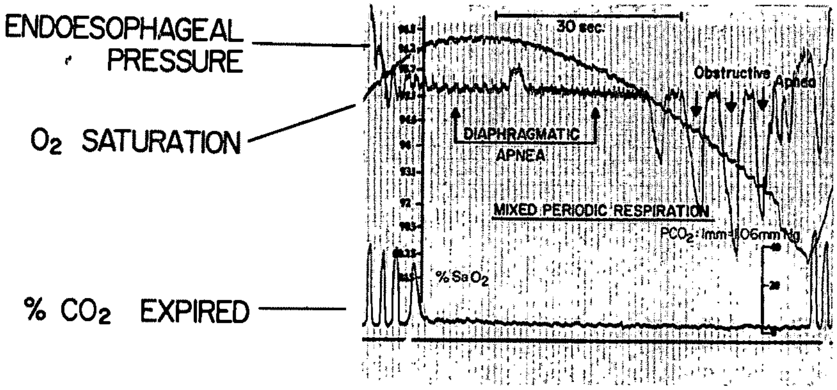

*Figure 1* Example of a sleep-induced mixed apnea recorded on a Sanborn polygraph. On the top tracing the endoesophageal pressure demonstrates the presence of a "central" or "diaphragmatic" component at the beginning of the apnea. No pressure change is recorded and there is no airflow through the upper airway, as demonstrated by the recording of expired $CO_2$ (*bottom tracing*). Diaphragmatic movements resume, and the first diaphragmatic swing produced normal pressure change (*top tracing*); however, no air passed through the upper airway (*bottom tracing*), and the second and third diaphragmatic swings produced larger endoesophageal pressure changes. This is due to a functional obstruction of the upper airway induced by sleep. In pure central apnea the recording is similar to the first part of the recording; in pure upper airway apnea the entire apneic period consists of increasing endoesophageal pressure swings and a flat expired $CO_2$ tracing. During the absence of air exchange, the blood oxygen saturation declines.

Although the sleep problem was often the most troublesome symptom for the patients and frequently resulted in the first medical consultation, we have found that the sleep disorder complaint represents only one of the clinical symptoms of the sleep apnea syndrome.

The following clinical descriptions have been derived from repeated questioning of our adult patients and their families, especially spouses of adult patients and parents of the younger patients. Some of these symptoms have been emphasized by other authors (28, 29).

### *Snoring*

In most patients the first clinical symptom was inordinately loud snoring. While many individuals with normal respiration during sleep may present light, intermittent nasal snoring, sleep apnea patients have loud pharyngeal snoring, associated with snorting and interrupted by silences (apneic periods) of 20 sec or longer. The history of this symptom can be very difficult to trace, but appears to be essentially lifelong in many patients. For example, in studies of infants considered "near miss" for the Sudden Infant Death Syndrome (30), two infants gradually developed abnormal snoring with apneic pauses during sleep. One child was 7 months old and the

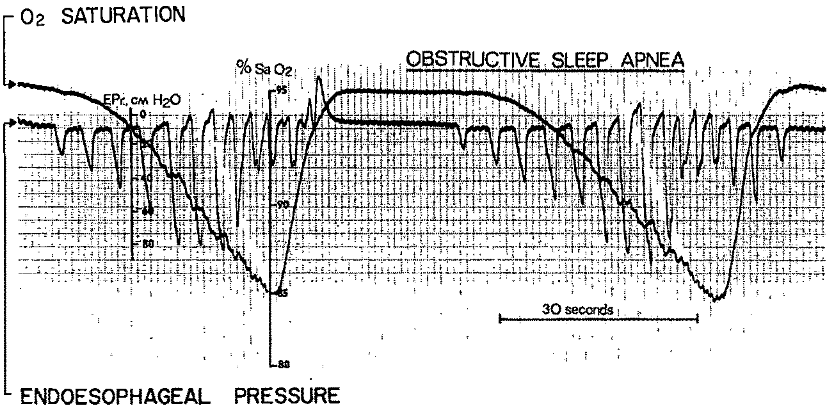

*Figure 2* Recording of successive apneic cycles during sleep (on a Sanborn polygraph) in a 42-year-old adult male complaining of excessive daytime sleepiness. The endoesophageal pressure demonstrates the intricate relationship of central and upper airway apneas in mixed apnea episodes. The changes in endoesophageal pressure reached values of nearly 80 cm $H_2O$ in association with the large diaphragmatic swings against a closed upper airway. Oxygen saturation drops during each apneic episode. There is a delay between resumption of normal respiration and oxygen resaturation, due to physiological components and also to the inertia of the recording system. The oxygen saturation returns to near normal values, but during the course of the night an alveolar hypoventilation syndrome will gradually develop with repetitive sleep-induced apnea.

other 12 months old when the snoring was first noted. In addition, the mother of a young boy with sleep apnea reported the onset of loud nighttime snoring when the child was 4 months of age. Parents of the other children in the Stanford sleep apnea population have reported observing abnormal respiration and snoring during sleep in the first two years of life.

In adult sleep apnea patients, the spouse or other bed partner is the best source of current information, and appropriate questioning always results in a vivid description of the loud snoring and unusual nocturnal respiratory pattern. In addition, patients commonly report that snoring has been a source of inconvenience and embarrassment for many years. Many patients are able to recall that family members and friends have expressed amazement or concern about their snoring from adolescence or early adulthood, long before the sleep complaint appeared.

In view of the ubiquity of snoring in sleep apnea patients, we feel that loud snoring, night after night, in children and adults even without an associated sleep complaint should strongly suggest a sleep apnea syndrome. Ear-nose-throat and all-night sleep evaluation should be considered in such instances.

### *Abnormal Behavior During Sleep*

It is apparent that abnormal behavior during sleep is a very prominent feature of the syndrome. Patients themselves seem to be generally unaware of these symptoms, which are reported only after specific questioning of the patient's spouse, bed partner, or family members.

Various abnormal movements are observed in association with repetitive sleep apneic episodes, occurring prior to resumption of respiration in each instance. These movements vary from a single, small movement of both hands or arms resembling a flapping tremor, to larger, more generalized movements of both upper and lower limbs. The movements can be extremely vigorous and in several cases injury to the bed partner has been reported. The spouses of several sleep apnea patients reported that they moved to a separate bed because they were unable to waken the patient and feared the large arm swings. In certain patients, movements include lifting the upper torso from the bed and then collapsing on the bed or the floor at the resumption of respiration (31).

Sleep apnea patients may also show somnambulism. This behavior generally appears after a series of repetitive apneic episodes, at which time the patient may arise and walk away from his bed, unresponsive to arousing stimuli. These episodes have been described by witnesses as ending abruptly when the patient collapses onto his bed or falls to the floor. Even after the patient falls to the floor, sleep is frequently uninterrupted and he may remain on the floor for the rest of the night. Injuries have been reported; one patient broke his wrist in this manner, and several bruised themselves repeatedly.

### *Altered States of Consciousness*

Laboratory observations have confirmed an altered state of consciousness during the night in sleep apnea patients. When arousal is attempted, patients are often unresponsive even to painful stimuli, and if the patient is finally aroused he typically exhibits temporal and spatial disorientation and difficulties with visual perception. Similar cognitive difficulties may also appear after waking in the morning, at which time patients complain of a "foggy mind" and an inability to remember events of the previous day. In children this abnormal state appears to be frequently associated with frightening hallucinations (31) that are not reported by adults, although they occasionally report "nightmares."

### *Nocturnal Enuresis*

In the Stanford series all children and three of the adults presented nightly enuretic episodes. One element appears to be peculiar to the nocturnal enuresis in all cases: bedwetting reappears after complete nocturnal toilet training. Complete urologic examinations were negative in those older children and adults who sought treatment for nocturnal enuresis prior to consulting the Sleep Clinic.

### *Morning Headache*

Approximately half of patients (both children and adults) with upper airway sleep apnea syndrome complain of repeated morning cephalgia, usually frontal, but sometimes diffuse. The headaches are present when patients awake in the morning and add to the "foggy" mind and general feeling of malaise. In general, the headaches dissipate several hours after arousal.

### *Sleep Complaints*

Daytime hypersomnolence is the usual reason that adult patients seek medical treatment. In children, however, hypersomnolence appears to be overlooked in spite of the heavy toll it exacts on learning and educational development. Adult patients complain of sudden, excessive drowsiness occurring inappropriately during the day and leading to an undeniable urge to sleep. They report falling asleep while driving, eating, talking, etc. The patients' lives and those of others are endangered because of these sudden sleep spells. For example, one patient in the Stanford series reported an incident that occurred while driving. He saw a railroad barrier closing about 300 yards ahead of him. The red blinkers were flashing, and he could hear the warning bell. The next thing he knew, his car had crashed into the barrier. Patients have also reported that daytime naps are not refreshing, but result in greater fatigue accompanied by "fogginess" and cephalgia.

Two additional symptoms are often reported in close association with daytime hypersomnolence. The first of these is an automatic behavior syndrome (32), characterized by complete retrograde amnesia lasting for as long as several hours. During such an episode it is possible for patients to complete simple, repetitive tasks, but complex behavior appears to be very difficult. This particularly includes the ability to speak coherently and appropriately. Polygraphic monitoring has revealed that frequent, repetitive microsleep episodes are directly related to the appearance and development of this syndrome (33).

Hypnagogic hallucinations are also commonly experienced by adult patients when the urge to sleep arises in the daytime. Hallucinatory images have included seeing "a tree in the road." "a flashing red light," or "a dog driving a car." Characteristically, the images persist for a short time until recognized as such by the patient and dispelled. However, patients often react before realizing their hallucinatory nature. For example, abrupt braking of a car to avoid "a tree" led to a serious accident in one of our cases.

Although not necessarily associated with the daytime hypersomnolence, sleep paralysis and cataplexy typical of the narcolepsy syndrome were reported by 11 adult patients.

Finally, in 14 cases in the Stanford series of sleep apnea patients, the chief complaint was of frequent and lengthy nighttime arousals, and many of the daytime symptoms were absent or less severe.

### *Physical Examination*

In the Stanford case series only 5% of the sleep apnea patients fit the description (12) of obese Pickwickian patients. In other cases series (34, 35) the percentage of Pickwickian patients has been much greater, presumably because the series did not originate in a sleep disorders clinic. Obesity and hypersomnolence, without the waking respiratory or cardiologic abnormalities, were seen in 34% of our patients. The remaining 61% were nonobese and without waking respiratory or cardiac symptoms. None of our patients showed the malformations that have been implicated in other alveolar hypoventilation syndromes, including micrognathia, kyphoscoliosis, functional atlanto-axio-occipital malformation (Dandy-Walker syndrome), and atlanto-axial dislocation (7, 8, 10, 36, 37). In all cases neurologic examination was normal, and EEG ruled out epileptic disorders. No endocrine disorders were found. The only significant finding from physical examination was elevated systolic and diastolic systemic blood pressure in 60% of the adults and 62% of the children. No specific causes could be found to explain this "essential" hypertension.

It should be reemphasized that the majority of patients with sleep apnea syndromes are *not* obese and have normal ventilation when awake. It is not likely that such patients will be referred to pulmonary specialists. We might infer, however, that such patients will begin to be identified in greater numbers in any center offering specialty evaluation during sleep for the complaints of daytime hypersomnolence and chronic insomnia.

### *Sleep Structure*

The data summarized below from the Stanford case series closely parallel the findings from other laboratories (35, 38–44). Apneic episodes during sleep are clearly related to a disruption of the ongoing sleep state. Most apneic pauses are associated with short EEG arousals characterized by the appearance of alpha rhythm and sometimes associated with slow eye movements, just prior to resumption of respiration. When a clear EEG alpha arousal is not present, a K complex usually signals the appearance of the disturbing stimulus. In addition to these fine disturbances of sleep, the overall structural staging of sleep is abnormal. In adults, stages 3 and 4 NREM (i.e. slow-wave) sleep are always significantly reduced or completely absent. On the other hand, the percentage of stage 2 NREM sleep during the night is abnormally high. REM sleep is generally present in normal amounts, although it is infrequently reduced to 15% of total sleep in certain patients. Sleep structure in children is similarly disturbed, with the exception of the youngest children with fewest sleep apneic episodes. In these cases the percentage of stages 3 and 4 NREM sleep is abnormally high, and these sleep stages are not associated with apneic pauses. Another sleep structure anomaly is the occurrence of REM sleep periods at sleep onset in approximately one fourth of the patients. This particular sequence of sleep staging is usually associated with narcolepsy (45), although it has also been observed in normal individuals after selective REM sleep deprivation and in circadian rhythm disturbances (46, 47).

## RESPIRATION DURING SLEEP IN NORMALS

To fully appreciate the respiratory abnormalities found during sleep in sleep apnea patients, we first review the findings from studies of normal subjects. Respiration has been studied in normal adults during REM and NREM sleep using a number of techniques. These include simple polygraphic recordings (25, 48–50) and polygraphic recordings correlated with either oxygen consumption (51, 52) or spirographic techniques (53–57). Unfortunately, as summarized by Duron (57), several technical problems may develop with certain of these recording procedures.

Nonetheless, clear respiratory irregularities during sleep have been noted in all the normal studies, and several general areas of agreement are apparent. Isolated, brief apneic episodes and spirographic abnormalities may appear normally at the onset of sleep and during REM sleep. Findings in our control population and those reported by Aserinsky (25, 49) showed that brief apneic pauses in REM sleep are generally associated with a burst of rapid eye movements. Respiratory pauses during sleep in normal adults are exceptional, and when they do appear they are not repetitive and rarely exceed 30 sec. Furthermore, in a study of 18 normal subjects (ages 45–60 years), respiratory irregularities during sleep were more frequent in men than in women. This finding is in accordance with the sex distribution of the sleep apnea syndrome, in which men outnumbered women by a ratio of 6:1 or 10:1 (19, 21, 34, 35).

Several investigators (58–60) have also reported increased carbon dioxide tension that appears to parallel sleep stages and seems to be associated with increased carbon dioxide threshold. In his normal subjects Bulow (55) distinguished two groups, based on carbon dioxide threshold during sleep. The first group was characterized by a very small, nonsignificant increase in carbon dioxide threshold during sleep; in the second group, the carbon dioxide threshold showed a large, significant increase during sleep. Although Bulow (55) characterized the second group as having "unstable" respiratory regulation, Duron (57) denied the development of significant hypercapnia during sleep in his subjects. Furthermore, Duron (57) stated that hypercapnia was probably not related to the specific issue of respiratory pauses during sleep, citing Fink et al (61), who showed that certain subjects discretely hyperventilate at sleep onset, possibly inducing the physiologic hypocapnia responsible for short apneic episodes at sleep onset. Two factors may have biased Duron's findings: first, he recorded only 12 subjects (compared to Bulow's 70 subjects), all of whom may have fallen into the first group described by Bulow. Second, Duron's subjects were all young adults (12–25 years). Although Bulow did not clearly state the age of the subjects in his "unstable" group, this factor may be significant, as indicated by the observations of Webb (51) and Webb & Hiestand (52), in which normal men over 50 were found to have significantly more respiratory irregularities during sleep than younger men.

The foregoing data suggest the possibility that there are two constitutionally different populations, one with regular respiration and similar $CO_2$ thresholds in waking and sleep, and the other presenting irregular respiratory functions during sleep. The suggestion that an underlying genetic predisposition for development of respiratory irregularities during sleep may exist is supported by a study in which Arkinstall et al (62) accounted for 80–90% of the variability in tidal volume response to $CO_2$ in terms of genetic factors. The existence of two such genetically different groups could have implications for the sleep apnea syndromes. It is possible that an unstable respiratory regulation during sleep could gradually develop into overt pathology. On the other hand, the unstable group might represent a population "at risk," in whom other factors, such as age, weight, mandibular position, and enlarged tonsils or adenoids might interact to produce a sleep apnea syndrome.

## RESPIRATION DURING SLEEP IN SLEEP APNEA PATIENTS

The typical sleep apnea patient has hundreds of repetitive or periodic apneic episodes in a single night. Depending upon the length of the sleep period and the duration of individual episodes, the usual range is between 200 and 500 (21, 63). Accurate determination of blood gases is extremely difficult because the levels are continuously changing. The peak and trough values of $PaO_2$ and $PaCO_2$ depend mainly upon the duration of an apneic episode and also upon the type of the apnea. It has become clear that $PaO_2$ drops more rapidly in upper airway sleep apnea than in central sleep apnea (21, 30).

A primary consideration in various studies has been to determine whether respiratory patterns during sleep differed among Pickwickian patients, obese sleep apnea patients, and nonobese sleep apnea patients (64). At present, the Stanford series comprises 23 adult male patients, whose sleep studies include blood gas determinations, ear oximetry, and precise quantitative measurement of respiratory variables. Table 1 summarizes sleep apnea variables in terms of the above three subgroups. As can be readily appreciated, there is no great difference in any value as a function of obesity or additional cardiopulmonary problem (e.g. Pickwickianism).

All patients showed repeated oxygen desaturation in association with apneic episodes. $PaO_2$ values did not differ among the groups. In the non-Pickwickian patients, alveolar hypoventilation was seen during sleep, and values quickly returned to normal when patients were fully awakened. During the night patients in all three groups presented cycles in which $PaO_2$ gradually changed from one series of repetitive apneic episodes to another. For example, during 10 or 15 min, the $PaO_2$ was 20–30 mm Hg at the end of an apnea and 30–40 mm Hg after resumption of respiration; this pattern might be followed by one in which, for no apparent reason (i.e. no change in sleep stage, body position, or pattern of apnea), the $PaO_2$ levels were 40–60 mm Hg at the end of an apnea and 55–85 mm Hg after resumption of respiration.

In REM sleep periods individual differences appeared, although these differences were independent of the patient group. In certain patients REM sleep was associated with a marked increase in the duration of apneic episodes from 90 to 130 sec. In other patients REM sleep periods were relatively free of apnea. Koo et al (65) have

**Table 1** Apneic episodes during sleep in patients[a]

| | Pickwickian | Obese, non-Pickwickian, sleep apnea | Nonobese sleep apnea |
|---|---|---|---|
| Number of patients | 3 | 10 | 10 |
| Mean number of apneas per night | 346 | 429 | 449 |
| Mean duration of apneic episodes (sec) | 25.0 | 27.2 | 29.1 |
| Mean apneas in NREM sleep (%) | 82.2 | 87.0 | 84.2 |
| Mean upper airway apnea (%) | 96.8 | 92.0 | 86.3 |

[a] Results based on averages from 8 hr of nocturnal sleep.

recently shown that patients with chronic obstructive pulmonary disease showed a worsening of arterial blood gas values, taken as an index of alveolar hypoventilation, in REM sleep. Similar results were seen in 15 of the patients included in Table 1, with no apparent relationship to patient group. These data, as well as a report by Lugaresi et al (41), suggest that obesity is not a requisite for severe sleep-induced apnea and that alveolar hypoventilation may develop to a similar degree during sleep in both obese and nonobese patients.

The relationship of changes in respiratory function to changes in sleep state suggests another factor that may be important in understanding the sleep apnea syndrome. As mentioned above, we have found certain patients in whom the respiratory irregularities are aggravated by REM sleep and others in whom respiration appears relatively normal in REM sleep. (A third group, uninfluenced by REM sleep, may also exist, but was not apparent in our sample.) The marked changes in respiratory pattern from wakefulness to sleep, and within sleep states themselves, underline the significance of brain mechanisms in the sleep apnea syndrome. Describing patient groups by these sleep state–related changes may provide more information than categories based on clinical findings such as obesity.

Another significant factor is the type of apnea patients present during sleep. In our sleep apneic patients all three types of apnea (central, upper airway, and mixed) are seen during each night, although the ratio of the types of apnea varies from patient to patient. There has been a tendency to assume that apneas involving diaphragmatic cessation result directly from a CNS defect, while upper airway apneas result from a peripheral obstruction. Several investigators (26, 42, 66, 67) have shown that upper airway sleep apnea may exist in patients in whom no cause of obstruction can be detected during wakefulness. In patients in the Stanford series who have had direct and indirect laryngoscopic examinations, no cause of obstruction was found in most cases. This may allow us to question the general role of physical obstruction: Why is upper airway sleep apnea present in only certain patients with micrognathia (10), obesity, Prader-Willi syndrome (68), and enlarged tonsils and adenoids (69–72)? Do CNS abnormalities interact with peripheral problems in these cases? Is a CNS defect the prime determinant of mixed and upper airway apneas that involve no peripheral factor? Are mixed apneas related to secondary hypoxia, hypercapnia, and acidosis, inducing a disruption of central respiratory regulation?

These questions cannot be answered conclusively, but suggestive evidence is available. Schwartz & Escande (73) demonstrated that a sudden "collapse" of the pharyngeal walls occurred during upper airway sleep apnea in Pickwickian patients. Using serial X rays, Smirne & Comi (74) found a slightly abnormal thickness and length of the soft palate of two Pickwickian patients during wakefulness. In sleep the pharyngeal wall collapsed slightly, interrupting the air column at the level of the soft palate between the base of the tongue and the pharyngeal walls. During upper airway apnea and associated with successive intrathoracic depression secondary to ineffective respiratory attempts, Smirne & Comi observed a progressive collapse of the lower portion of the pharyngeal walls and the upper pharynx. We attempted to abort the upper airway apnea by positioning a pharyngeal tube during the night in a small series of four patients. Only one patient showed any change at all as a result of this approach. Direct electromyographic recording of muscles such as the middle constrictor of the pharynx may give more clues to the mechanisms underlying upper airway apnea. The likelihood of CNS involvement in all three types of sleep apnea seems reasonable at this time.

There is some evidence that the secondary hypoxia, hypercapnia, and acidosis may aggravate the perturbation of respiratory function during sleep. One of the most effective treatments of sleep apnea syndromes involving a predominance of upper airway apnea is the insertion of a permanent tracheal valve, closed during the day and opened at night to bypass the pharyngolaryngeal problem (18, 21, 75–77). One would assume a priori that such a procedure, while eliminating upper airway apnea, would have little effect on pure central apnea or the diaphragmatic (central) component of mixed apneas. Recordings conducted on nine patients (two children and seven adults) at four weeks following tracheostomy showed no upper airway apnea, although central apneas continued. On subsequent recordings conducted up to 12 months following surgery there was a significant decrease in central apneas during sleep. These data suggest the presence of a feedback loop in which impairment of the respiratory drive leads to sleep-induced apnea; resultant hypoxia, hypercapnia, and acidosis interact secondarily with the "respiratory neuronal network" (in its very broadest meaning) to increase the possibility of disturbance in CNS respiratory regulation.

## WAKING RESPIRATORY FUNCTION IN THE SLEEP APNEA SYNDROMES

Respiratory abnormalities in Pickwickian patients have been reported extensively (15, 38, 40, 78). In collaboration with Drs. Fred Eldridge and James Phillips at Stanford, we have evaluated waking respiratory function in six nonobese and five obese adult sleep apnea patients. Some of these essentially negative data have been reported (21). Pulmonary function tests included spirometric tests, studies of lung volume, elastic recoil, flow resistance, and arterial blood gases. An exercise study compared rest and three grades of exercise. $CO_2$ response curve and post-hyperventilation breathing tests as described by Plum et al (79) were also conducted. During full alertness these tests were all within normal limits, but rapid deterioration of values was noted when patients became drowsy. Without proper evaluation of the patient's state of vigilance, sleepiness may produce erroneous interpretation of daytime pulmonary evaluations in these patients.

## SLEEP COMPLAINT AND PREDOMINANT TYPE OF SLEEP APNEA

In the Stanford case series the majority of patients showed a predominance of upper airway sleep apnea associated with a complaint of excessive daytime hypersomnolence (21, 23). In the remaining patients there was a predominance of central apnea associated with the complaint of daytime fatigue and disturbed nocturnal sleep (insomnia) (63, 80). Patients in the latter group did not snore continuously, but heavy snoring was always present at some point in the night. The sleep of these patients was characterized by an essentially normal sleep latency, several lengthy nocturnal arousals, and premature morning awakenings. Hypoxia and hypercapnia were less pronounced than in patients with upper airway apnea. (Differences in oxygen consumption due to muscular effort may explain this finding.) Hemodynamic changes were also less marked in these patients.

## HEMODYNAMICS IN NORMAL SLEEP

### *Arterial Pressure Changes During Normal Sleep*

Using indirect methods, Snyder et al (48, 81) and Richardson et al (82) have shown that significant hemodynamic changes occur during sleep in normal human volunteers. Khatri & Freis (83), Bristow et al (50), and Coccagna et al (84) generally confirmed these earlier reports by cannulating the brachial artery for direct, continuous recording of the arterial pressure during sleep in association with standard sleep and respiratory recording parameters.

These latter investigators showed that the arterial pressure decreases rapidly during the first hour of sleep and gradually increases in the second part of the night. All three reports agreed that there is a direct relationship between sleep stages and arterial pressure; the lowest systolic and diastolic values were noted in stages 3 and 4 NREM sleep. Bristow et al (50) also reported very low values in REM sleep, but this finding was not confirmed by either Khatri & Freis (83) or by Coccagna et al (84). Snyder et al (48, 81) reported consistently higher values of arterial pressure in REM sleep than in stages 3 and 4 REM sleep, sometimes exceeding even the highest values recorded in the same subject during the waking hours. This finding was confirmed by Coccagna et al (84), who also found that the arterial pressure values recorded during the early part of sleep (NREM) were significantly lower than the values recorded during wakefulness. The results of Khatri & Freis (83) and Bristow et al (50) did not show a statistically significant decrease. Finally, Coccagna et al (84) speculate that an ultradian variation of arterial pressure may be superimposed on the pressure variations linked to sleep stages.

### *Pulmonary Arterial Pressure Changes During Normal Sleep*

There is only one study of pulmonary arterial pressure (PAP) changes during sleep in normals. Lugaresi et al (85) continuously monitored PAP in three normal subjects by means of a microcatheter positioned in the pulmonary artery using Grandjean's technique (86). Polygraphic recording of EEG, electro-oculogram (EOG), chin electromyogram (EMG), ECG, and respiration was conducted simultaneously. Cyclic variations of PAP with a period of approximately 20–30 sec were noted during states 1 and 2 NREM sleep. The variation in PAP reached a maximum of 2–4 mm Hg above the baseline value obtained during wakefulness. Stages 3 and 4 NREM sleep seemed to be the most stable periods, with no significant PAP fluctuations (E. Lugaresi, personal communication, 1973).

## HEMODYNAMIC AND CARDIAC ABNORMALITIES IN SLEEP APNEA SYNDROMES

Right heart hypertrophy and congestive heart failure are considered part of the Pickwickian syndrome. Similarly, chronic nasopharyngeal obstruction or adenoid and tonsillar hypertrophy can lead to cor pulmonale in children (69–72, 87, 88). From nocturnal recordings of pulmonary and systemic arterial pressure, Coccagna et al (89) suggested that sleep-induced apnea with hypersomnia ("hypersomnia with periodic breathing") occurring regularly during sleep over a period of years, may be responsible for the development of cor pulmonale. We have also continuously monitored pulmonary arterial pressure (using a Swan-Ganz ® catheter) during 16–18 hr in insomniac patients with sleep-induced apnea (80, 90) and in nonobese hypersomniac patients with sleep-induced apnea (21). Recently, in collaboration with Drs. Ara Tilkian and John Schroeder, we performed more complex monitoring in 12 sleep apnea patients who were obese but did not show other characteristics of the Pickwickian syndrome (91). Fifteen hours of continuous monitoring, beginning in the late afternoon, included pulmonary arterial pressure, systemic arterial pressure, and $PaO_2$ with a femoral arterial oxygen electrode (in addition to standard sleep parameters and respiratory variables). Prior to the continuous monitoring, intracardiac electrophysiologic studies were performed. These included His bundle recordings, atrial pacing, and sinus node recovery time. Cardiac output determinations were made during sleep after the onset of hemodynamic abnormalities, and blood gases were measured intermittently throughout the day and night. One or two continuous 24-hr periods of ECG monitoring (Holter recording) were also carried out.

The data from Stanford are concordant with the findings of Coccagna et al (75); that is, significant hemodynamic abnormalities developed during nocturnal sleep secondary to the repetitive apneic episodes. Such abnormalities were directly related to the total number of sleep apneic episodes during the night.

Increased pulmonary arterial pressure appeared with the vigorous inspiratory efforts performed against a closed airway during upper airway sleep apneic episodes. Very large intrathoracic pressure swings developed, ranging from 5 to −85 mm Hg. At the onset of ventilation, these pressure swings immediately dropped to a normal range, but rises in pulmonary arterial pressure were typically sustained. It should be noted that pulmonary arterial pressure was also elevated in insomniacs with central sleep apnea (63). Coccagna et al (89) reported a correlation of sleep stages with PAP and systemic pressure, including a gradual increase from wakefulness to stages 1–4 NREM sleep and a great rise with REM sleep. In our patients the increase in arterial pressure was closely related to the distribution of apneic episodes. In patients who presented no sleep apnea during REM sleep, PAP measurements were almost normal at that time. The onset of rises in systemic pressure occurred following the lowest point of oxygen saturation of an apneic cycle at the onset of ventilation and sinus tachycardia. In several patients cardiac output was determined at various intervals. No significant changes were seen in cardiac output (A. Tilkian et al, in preparation). Continuous ECG monitoring (Holter recording) showed a variety of arrhythmias, some potentially life threatening, during the night in association with repetitive sleep apneas (91). Episodes of sinus arrest, asystoles up to 6.4 sec, heart block, and ventricular tachycardia were seen. No arrhythmias were seen during wakefulness. In 50 cardiac patients (without sleep apnea) who had undergone similar electrocardiographic recording, none presented these arrhythmias, associated only with nocturnal sleep (91). Kryger et al (44) also noted atrial flutter fibrillation with a slow ventricular response and prolonged asystole during sleep-induced apnea in one patient.

We have already mentioned the very important observations of Lugaresi et al (29), who found that eight normal volunteers who were "heavy" snorers all showed a typical sleep apnea syndrome with associated hemodynamic abnormalities.

## COMMENTARY

Historically, it is understandable that the most complete form of sleep apnea syndrome, reported by Burwell et al (12), was the first to be recognized as a pathological entity. We now recognize, however, that most of the symptoms reported by Burwell et al (12) are secondary to an abnormal respiratory pattern during sleep. Depending on the type, frequency, and intensity of the abnormal sleep-related respiratory pattern, the clinical symptomatology will differ. Abnormalities induced by a developing sleep apnea syndrome are first done during sleep, and only gradually affect the waking period. The time course of the illness depends on several factors, including the severity of the nocturnal respiratory problem, the patient's age, the presence of obesity, upper airway obstruction, and so forth. The sequence in which symptoms appear may also vary. For example, we have seen one patient with an upper airway sleep apnea syndrome who shows cardiac arrhythmia, hypertension, and increased pulmonary arterial pressure with each repetitive apnea. Although this patient has developed "essential" hypertension during wakefulness and is progressively developing right ventricle impairment, his weight is entirely normal and he is without daytime hypersomnolence. Other patients present profound daytime sleepiness, but no other clinical findings, and a variety of other combinations may be found. The common factor in all patients, however, is the sleep-induced apnea syndrome.

Although many questions about the sleep apnea syndromes remain unanswered, a few points are now very clear. These include the role of sleep-induced apnea as a primary cause of multiple clinical symptoms, the relationship of a Pickwickian syndrome to the evolution of a sleep apnea syndrome, and the important therapeutic role of chronic tracheostomy that bypasses the upper airway obstructions and protects the patient from the secondary deleterious clinical symptoms. This treatment was first reported by Kuhlo et al (18) and Coccagna et al (75). The Stanford case series of upper airway sleep apnea patients now includes 12 patients successfully treated with this approach by Dr. F. Blair Simmons (21, 67).

One interesting problem for sleep researchers is why patients complain of fatigue and daytime somnolence. Two schools of thought have arisen from sleep studies in Pickwickian patients: The first suggests that the hypersomnolence is secondary to an accruing sleep deprivation caused by repeated disturbance of sleep by the respiratory difficulties (15, 35, 66); the second suggests that the hypersomnolence may result from a defect of the CNS structures that control sleep (10, 22, 66). Recent sleep studies of nonobese sleep apnea patients with and without excessive daytime sleepiness, and obese sleep apneics without daytime sleepiness, have called into question both hypotheses, chiefly because similar abnormalities of sleep structure have been found in all cases.

Further knowledge derives from the finding that hypersomnolence disappears within 24 hr after tracheal intubation or permanent tracheostomy, either of which resolves the respiratory abnormality and consequent sleep disturbance. This finding seems to rule out a purely central origin of the hypersomnolence. On the other hand, the concept of hypersomnia resulting solely from the sleep disturbance is difficult to reconcile with recent findings from heavy snorers with sleep apnea who present no daytime sleepiness (29) and from two patients in our population who presented a typical sleep-induced apnea syndrome with no daytime complaints. In addition, we have also studied ten patients in whom closure of tracheostomy for one night instantaneously produced reappearance of the daytime symptoms of such severity as is never seen in normals who undergo a full night of sleep deprivation. Lugaresi and his colleagues (66) have suggested that the nocturnal hypercapnia and continuous oscillation in the $PaCO_2$ that result from repetitive sleep apneic episodes may "alter the reactivity of the reticular activating system" in such a way as to affect daytime vigilance. We feel that simultaneous changes in other blood gases should also be considered.

While it appears certain that sleep apnea, disturbed nocturnal sleep, and excessive daytime sleepiness are related, the exact mechanisms that lead to the daytime sleepiness are not clear. The symptom of daytime hypersomnolence apparently develops gradually during the course of the disease, and the available data do not support the conclusion that any factor is wholly responsible. The hypoxemia, hypercapnia, and acidosis that occur during sleep are not sufficient to explain the hypersomnolence, since patients with chronic lung disease and chronic alveolar hypoventilation do not necessarily present this symptom. In addition, Cole & Alexander (14) reported hypersomnia in obese patients with no carbon dioxide retention.

In our opinion, the daytime hypersomnolence in sleep apnea patients may result from the fluctuating nature of all these changes. In the sleep apnea syndromes it is clear that blood gas values fluctuate repeatedly and abruptly many times throughout the night. Thus the patients are in a very *unstable state* during sleep, in contrast to normal individuals, or even patients with continuous alveolar hypoventilation in whom there is a *stable state,* either normal or pathological. Metabolic changes induced by the unstable state in sleep apnea may be of greater significance and severity than those seen in the pathological, but stable state.

Other factors that have received little attention include the impact of the blood gas changes on neurotransmitters in the brain and the effects of the hemodynamic changes on cerebral blood flow. Hypoxia may interact with monoamine synthesis (92) and may significantly increase the cerebral content of $\gamma$-aminobutyric acid as well (93). Thus, alteration of neurotransmitters involved in sleep processes may also play a role in the daytime hypersomnolence.

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# INTERRELATIONS OF RENIN, ANGIOTENSIN II, AND SODIUM IN HYPERTENSION AND RENAL FAILURE

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## INTRODUCTION

Although renin was isolated from normal rabbit kidneys by Tigerstedt & Bergman in 1898 (1), and Goldblatt first produced experimental hypertension in 1934 (2), the large number of studies by many investigators during the past 20 years has documented the importance of the renin-angiotension system in normal cardiovascular and electrolyte physiology and in the pathogenesis of a variety of human diseases. It is currently recognized that the renin-angiotensin system plays a major role in the homeostasis of sodium, potassium, and water, and in the control of arterial blood pressure. Abnormalities of the system are apparent in several forms of human hypertension, edematous states such as congestive heart failure and renal failure. The purpose of this article is to survey the recent medical literature concerning: (*a*) the role of the renin-angiotension system in normal physiology, and (*b*) the role of the system, together with sodium, in the pathogenesis of hypertension and renal failure. The survey is selective and limited. The focus is on information that may assist the clinician in understanding the role of the renin-angiotensin system in normal homeostasis and in the interpretation of pathophysiological mechanisms.

## THE RENIN-ANGIOTENSIN SYSTEM

Renin is a proteolytic enzyme produced and secreted by the juxtaglomerular cells, which are located in the walls of the afferent arterioles of the kidney adjacent to the glomeruli (3, 4). Renin acts upon a circulating α-globulin synthesized by the liver, the renin-substrate (also called angiotensinogen), to split off a decapeptide, angiotensin I ($A_I$). Angiotensin I has little biological activity. However, an enzyme present primarily in the lungs and also in circulating plasma, blood vessels, and the kidney (converting enzyme), splits off two additional amino acids to form biologically active angiotensin II ($A_{II}$). Since, under normal conditions, neither substrate nor converting enzyme concentration is rate limiting, the amount of circulating renin is the major determinant of $A_{II}$ production. $A_{II}$ constricts vascular smooth muscle, stimulates aldosterone secretion by the adrenal cortex, and promotes sodium ($Na^+$) retention by the kidney. It is currently thought to be the primary effector hormone of the renin-angiotensin system (4). Recent studies, however, have revealed the existence in plasma of peptide subunits of the $A_{II}$ molecule (5). The infusion of one of them, the heptapeptide des-asp-angiotensin II (called angiotensin III), results in no change or a slight rise in arterial pressure and significant increase in aldosterone secretion (6). The precise role of the $A_{III}$ in normal homeostasis and disease is uncertain at this time.

Present knowledge of the control of renin secretion by the kidneys has been summarized in recent review articles (3, 4, 7). Many factors appear to be important. The level of arterial blood pressure sensed by baroceptors located in the afferent arteriole is a major factor. Low renal artery pressure stimulates renin secretion by the kidneys (8). The sympathetic nerves that terminate among the juxtaglomerular cells and the adrenal catecholamines also stimulate renin release, probably via a β receptor in the juxtaglomerular cell region (9–11). There also appears to be feedback inhibition of renin secretion by circulating $A_{II}$ and by plasma potassium. Infusions of $A_{II}$ suppress renin secretion, whereas infusions of agents that inhibit the generation or action of $A_{II}$ result in significant increases in plasma renin (12–15). Hyperkalemia suppresses renin release, whereas hypokalemia stimulates renin output by the kidneys (16–19). Finally, it is also believed that some function of the delivery of sodium choloride to the distal nephron, or of the transport of these ions in the distal tubule, is sensed by the macula densa cells and influences renin secretion (20). The macula densa cells occur in the portion of the distal tubule that abuts on the vascular pole of the glomerulus. They are contiguous with the juxtaglomerular cells of the afferent arteriole. With the juxtaglomerular cells and the mesangial cells of the glomerular stalk, they form what has been termed the "juxtaglomerular apparatus." Changes in peripheral renin levels have been observed in animals in which the delivery of sodium in tubular fluid to the distal nephron or reabsorption at the macula densa was altered by a variety of methods (20–23). However, changes in renin secretion have also been induced by hemorrhage or aortic constriction in dogs with nonfiltering kidneys, in which macula densa function was absent (8).

## THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN HOMEOSTASIS

It has become increasingly apparent that the renin-angiotensin system is involved in the maintenance of arterial blood pressure, control of extracellular fluid volume, and regulation of electrolyte excretion by the kidney. Its role in the control of blood pressure is complex, involving both peripheral vasoconstrictor mechanisms and adjustments of sodium and potassium excretion by the kidney. For convenience, three topics are reviewed separately: (*a*) the vasoconstrictor influences of the renin-angiotensin system, (*b*) the system's influence on the control of sodium and water balance via the adrenal gland, and (*c*) its effects upon renal function. This is followed by a discussion of the interactions between the renin-angiotensin system and sodium that occur in hypertensive diseases and in renal failure.

### *Vasoconstrictor Action of Angiotensin II: Role in Normal Blood Pressure Control*

In an oversimplified view, the level of arterial blood pressure is determined by (*a*) the cardiac output, (*b*) the volume of fluid distending the arterial vascular tree, and (*c*) the vasomotor tone of the vessels that determine the resistance to blood flow. The vasomotor tone is determined, in turn, by a variety of metabolic and hormonal factors. Known vasconstrictor factors include the sympathetic nervous system, the adrenal catecholamines, and $A_{II}$. Known vasodilator influences include the kallikrein-kinin system and the prostaglandins.

$A_{II}$, on a weight basis, is the most powerful pressor substance known (4). Although $A_{II}$ has an indirect pressor effect by an action on the central nervous system (CNS) (24, 25) and by an action to enhance catecholamine release from sympathetic nerves and the adrenal medulla (26, 27), the increase of arterial blood pressure results primarily from a direct effect of the octapeptide that causes contraction of vascular smooth muscle. It has also been established that angiotensin elicits a positive inotropic response in isolated myocardium (28, 29). This effect is independent of sympathetic nerve stimulation and is probably mediated by angiotensin receptors in the cardiac tissue (30). The significance of these findings is not yet clear, however. In studies of animals and man, the rise in blood pressure induced by $A_{II}$ is accompanied by an increase in peripheral resistance with either no change or a fall in cardiac output (31, 32).

In the 1960s it was found that increased levels of circulating renin (and of $A_{II}$) were associated with elevations of arterial blood pressure in severe and malignant essential and renovascular hypertension in experimental animals and man (3, 4, 33–36). Nevertheless, equally high levels of circulating renin were found in a variety of conditions in which the blood pressure was not elevated. These included dietary sodium depletion (37), hemorrhage (8), pregnancy (38), cirrhosis with ascites (39), nephrosis (40), clinical and experimental congestive heart failure (41, 42), and in the syndrome of juxtaglomerular cell hyperplasia (43). The reason for the different levels of blood pressure in the different states associated with high plasma renin activity was unclear.

Studies in which infusions of $A_{II}$ were administered indicated that sensitivity to the pressor effects of the octapeptide varies extensively in the different situations. The pressor response to $A_{II}$ infusion in normal animals or man is reduced by prior salt depletion (42, 44, 45), hemorrhage (4), or adrenalectomy (46); it is enhanced by a positive sodium balance in animals (45) and also by the positive sodium balance that develops in man during chronic infusions of $A_{II}$ (44). The pressor response to $A_{II}$ is also reduced in patients with cirrhosis and ascites (44), in nephrosis (47), in animals with partial ligation of the thoracic inferior vena cava (TIVC), an experimental model of congestive failure, (42), in renovascular and malignant hypertension (48), and in patients with the syndrome of juxtaglomerular cell hyperplasia (43).

The recent availability of pharmacological and immunological agents that inhibit the vasoconstrictor effects of $A_{II}$ has stimulated experiments that provide insight into these differing observations. Johnson & Davis infused a competitive analogue of $A_{II}$, 1-sarcosine, 8-alanine $A_{II}$ (Saralasin®), intravenously into normal and sodium-depleted dogs and into dogs with TIVC ligations (14,15). While $A_{II}$ blockade with Saralasin produced no change in blood pressure in the normal animals, significant reductions of pressure were observed in the sodium-depleted and TIVC dogs, along with reductions in aldosterone secretion and increases in plasma renin activity (14, 15). The data suggested an important role for $A_{II}$ in the maintenance of normal blood pressure via its action on specific receptor sites in arteriolar smooth muscle. These observations have been confirmed by other groups in rats (49, 50), rabbits (51), and man (52), using either the peripheral $A_{II}$ inhibitor Saralasin, or an inhibitor of the enzyme that converts $A_I$ to $A_{II}$, converting enzyme inhibitor (CEI). In addition, reductions of arterial pressure were observed when blockers of $A_{II}$ action were administered to animals with a stable degree of hemorrhage (53), advanced chronic TIVC occlusion, or experimental right heart failure (54). Since the blood pressure reductions during the infusions occurred rapidly, they could not be attributed to alterations of aldosterone secretion or to changes in sodium balance. The studies indicate that the renin-angiotensin system plays an important role in the defense of arterial blood pressure whenever the effective filling of the systemic arterial tree is threatened. In sodium-replete normals, inhibition of $A_{II}$ does not lower arterial blood pressure. Thus it appears that during sodium depletion, TIVC occlusion, hemorrhage, or heart failure, arteriolar vasoconstriction mediated by $A_{II}$ contributes significantly to the maintenance of normal blood pressure. This homeostatic role is unmasked by the administration of agents that either inhibit $A_{II}$ generation or compete with it for receptor sites on the arterioles.

The studies with inhibitors of $A_{II}$ have not fully explained the mechanisms responsible for the alterations of pressor sensitivity that have been observed during infusions of exogenous $A_{II}$, or the phenomenon that high-renin states are not always associated with an elevated blood pressure. Other investigators have focused on the interactions between $A_{II}$ and hormone receptors on vascular smooth muscle cells. Brunner and co-workers postulated that the affinity of vascular $A_{II}$ receptors could be influenced by the state of sodium balance (55). In their study, the amount of antibody against $A_{II}$ required to block the pressor effect of infusions of exogenous $A_{II}$ was used as an index of vascular receptor affinity to $A_{II}$ under varying conditions of sodium intake and in several models of hypertension. The amount of antibody required to block $A_{II}$ was greater in high-salt than in low-salt rats, in rats treated with desoxycorticosterone and salt, and in those with chronic renal hypertension.

Since sodium balance influenced the amount of antibody required to block $A_{II}$ in nephrectomized animals that had no circulating $A_{II}$, they concluded that the changes in apparent vascular affinity for $A_{II}$ were induced by changes in sodium balance. According to the scheme proposed by these workers, renin and $A_{II}$ concentrations increase during sodium depletion, but vascular affinity for $A_{II}$ falls, so blood pressure remains normal. Conversely, a sodium surfeit depresses renin and $A_{II}$, but enhances pressor effects of the circulating polypeptide.

An alternative explanation for the decreased pressor effects of exogenous $A_{II}$ infusions during $Na^+$ depletion, proposed by Davis and his co-workers more than 12 years ago (42), is that during sodium depletion, vascular receptors are occupied by endogenous $A_{II}$ and are unavailable to exogenous hormone. To test this hypothesis, Thurston & Laragh measured the pressor responses to $A_{II}$ infusion in rats before and after inhibition of endogenous $A_{II}$ generation by administration of CEI (50). In sodium-deprived rats, $A_{II}$ increased blood pressure to a smaller extent than in sodium-loaded or nephrectomized rats, and CEI lowered resting pressure only in the former animals. However, the important observation was that the pressor responses to $A_{II}$ in the sodium-depleted rats were identical to those of the sodium-loaded rats after endogenous $A_{II}$ generation was blocked with CEI. Since the restoration of vascular reactivity to $A_{II}$ in these acute studies could not be attributed to alterations of sodium balance, these investigators concluded that differences in pressor sensitivity to $A_{II}$ probably resulted from competition between exogenous and endogenous hormone for the same vascular receptor sites. During CEI administration, more receptors may become available, so that pressor effects of exogenous $A_{II}$ are more marked. In this regard, endogenous $A_{II}$ need not mean only circulating polypeptide. Effects of sodium depletion on the reactivity of aortic strips to $A_{II}$ have been demonstrated in vitro (56). Other evidence suggests that renin and renin substrate may accumulate locally in arteriolar walls and may lead to local generation of $A_{II}$ (57).

From the preceding it is clear that new information is being obtained concerning the vasoconstrictor action of $A_{II}$ upon peripheral arterioles and concerning the role of $A_{II}$ in normal blood pressure regulation. However, it is also becoming apparent that the net level of peripheral arteriolar tone may be a summated effect not only of vasoconstrictor factors, but also of a variety of vasodilator influences. Recent studies have called attention to the renal prostaglandins as a possible vasodepressor, vasodilator system (58). In the kidney $A_{II}$ infusions produced vasoconstriction and also increased synthesis of the vasodilator $PGE_2$, which counteracted the flow-reducing effect of the pressor octapeptide (59). $PGE_2$ synthesis was also demonstrated in isolated mesenteric vessels (60) and endothelial cells cultured in vitro (61). Enhanced circulating levels of a substance immunoreactive to antibodies raised to PGA (another prostaglandin vasodilator) were reported in man during $Na^+$ depletion (62). In dogs, hemorrhage and hypotension were associated with the appearance in arterial blood of PGE-like material, and the subsequent administration of indomethacin, an inhibitor of prostaglandin synthesis, caused a rise in blood pressure (53). Finally, parallel changes in renin, $A_{II}$, and plasma levels of bradykinin, a potent vasodilator, were recently reported in man during sodium depletion and sodium loading (63). A discussion of the prostaglandins and of the kallikrein-kinin system exceeds the scope of this review. It is possible, however, that the vasodilator factors co-participate with the renin-angiotensin system in the normal control of blood pressure and tissue perfusion; they may also play a role in determing vascular sensitivity to $A_{II}$.

### *Angiotensin II–Adrenal Interactions in Control of Sodium and Water Balance*

The work of many investigators in the 1960s clearly established that $A_{II}$ stimulates the secretion of aldosterone by glomerulosa cells of the adrenal cortex in virtually all mammalian species (4). Aldosterone, in turn, promotes the tubular reabsorption of sodium and the secretion of potassium in the distal portions of the mammalian nephron. A variety of studies in normal animals and man, summarized elsewhere, have found close correlations between the state of sodium balance, plasma renin activity, and the rate of aldosterone secretion (4). Sodium depletion enhances secretion of both renin and aldosterone, and sodium administration has opposite effects. Serial measurements of renin activity, $A_{II}$ and aldosterone in plasma have also supported the current belief that changes of aldosterone secretion in response to alterations of sodium balance are mediated primarily by the renin-angiotensin system (64). This conclusion is supported further by recent observations that in sodium-depleted or TIVC dogs, and in high-renin hypertensive patients, infusions of the angiotensin inhibitors Saralasin or CEI were associated with acute reductions of plasma aldosterone (14, 15, 65).

Other factors also exert major influences upon adrenal aldosterone secretion. Increases in plasma potassium concentration and in potassium balance stimulate aldosterone secretion in normal animals and man independently of the renin-angiotensin system; potassium depletion has opposite effects (66). ACTH exerts direct stimulating effects that are transient (67). Whether hyponatremia has an independent direct stimulatory effect in man is unclear. Searches for a pituitary substance controlling aldosterone secretion have been elusive. In sum, the renin-angiotensin system appears to be a major control system for aldosterone release in normal human physiology. However, a variety of studies suggest that potassium or other factors may also assume a major importance in diseases such as advanced renal failure (68, 69).

Of possible significance to the role of the renin-angiotensin system in the control of aldosterone secretion in hypertensive states (particularly low-renin essential hypertension) are recent studies that indicate that the adrenal sensitivity to $A_{II}$ may vary during alterations in sodium balance. Although some of the evidence is conflicting (70), two groups have provided data in normal man indicating that when sodium restriction reduces the pressor effects of $A_{II}$ infusion, it simultaneously potentiates the adrenal aldosterone secretory response to the polypeptide (71, 72). The explanation for this phenomenon, which may involve alterations in the binding of $A_{II}$ to adrenal cells (73) or changes in the rates of adrenal biosynthetic reactions, is not known.

## *Direct Effects of Angiotensin II on Renal Function*

SODIUM EXCRETION Infusions of $A_{II}$ in normal animals and man result in acute reductions of the urinary excretion rates of $Na^+$ and water, which precede changes mediated by aldosterone (74–77). In one preliminary study in which $A_{II}$ was infused into the renal artery of salt-loaded dogs, a decline in $Na^+$ output was observed with no significant changes in glomerular filtration rate (GFR) or renal plasma flow (RPF) (78). Although a direct effect of $A_{II}$ to enhance renal tubular $Na^+$ reabsorption was postulated (78), the data in this study differ from other clearance studies, in which reductions of RPF were observed during intrarenal infusions of $A_{II}$ (74–77). Furthermore, micropuncture studies in rats have failed to demonstrate any direct effects of $A_{II}$ on absolute proximal tubular sodium or fluid reabsorption (79, 80).

In marked contrast to the sodium retention observed in normal subjects during $A_{II}$ infusions, paradoxically large natriuretic and diuretic responses have been observed in patients with cirrhosis and ascites (74). This phenomenon and the smaller natriuresis that occurs in some hypertensive patients, rats, and TIVC dogs during $A_{II}$ infusions remains unexplained; it may result from subtle changes in intrarenal hemodynamics (74–77).

RENAL BLOOD FLOW Intravenous infusions of $A_{II}$ induce renal vasoconstriction in animals and man; there are proportionally larger reductions in renal blood flow (RBF) than in GFR, so that filtration fraction rises (74–77). In an elegant micropuncture study in rats, Myers, Deen & Brenner showed that $A_{II}$ infusions caused vasoconstriction of the efferent arterioles, which reduced glomerular plasma flow (79). Because the oncotic pressure in the efferent arterioles rose to an extent that equaled the increase in the glomerular transcapillary hydraulic pressure difference, which was brought about by the $A_{II}$ infusion, filtration pressure equilibrium was maintained and single-nephron glomerular filtration rate did not change. Absolute proximal tubular reabsorption was not altered by $A_{II}$ infusions in these studies (79).

Evidence has also been reported that implicates $A_{II}$ in the changes in renal blood flow resulting from alterations in sodium balance. RBF declines in response to sodium deprivation in both animals and man (51, 81). Studies using $^{133}Xe$ and radioactive microspheres indicate that this decline is proportionately greater in the outer renal cortex (51, 71, 81). Infusions of exogenous $A_{II}$ in sodium-depleted animals and man diminished RBF to a lesser extent than during $Na^+$ repletion (71, 74, 82). In sodium-depleted rabbits, infusions of the $A_{II}$ inhibitors Saralasin or CEI restored the depressed RBF almost to normal levels despite a concomitant fall in blood pressure; greater flow increases occurred in the outer cortex (51). Similar results have been obtained by Freeman et al in sodium-depleted dogs and in TIVC dogs (83). Interestingly, the renal vasodilatation induced by inhibition of $A_{II}$ in all of these experiments was not accompanied by a significant rise in urinary sodium excretion (51, 83).

INTRARENAL CONTROL OF GLOMERULAR FILTRATION RATE The Belgian pathologist Goormaghtigh, who first described the macula densa and juxtaglomerular cells in 1932, suspected that there might be a feedback control mechanism between the distal tubule and glomerulus because of the anatomic proximity of these structures (84). Thurau and his co-workers (85, 86), in the 1960s, postulated that an increased concentration of $Na^+$ in the distal tubule sensed at the macula densa might increase renin release by juxtaglomerular cells; local formation of $A_I$ and $A_{II}$ would result in constriction of the adjacent afferent arteriole and thereby reduce blood flow and GFR in the same nephron (86).

Although unproven and controversial, this theory has provoked additional experimentation. Renin converting enzyme and angiotensinases have been detected in microdissected juxtaglomerular apparatuses of the rat (87). Angiotensin II has also been found in renal interstitial fluid and lymph in concentrations exceeding that of blood; this finding implies intrarenal formation of $A_{II}$ (88). In addition, $A_I$ infused into the renal artery underwent conversion to $A_{II}$ within the kidney (89).

Other studies, however, have demonstrated that autoregulation of RBF and GFR is not impaired during constant infusions of an excess of $A_{II}$ (90) or when the intrarenal generation of $A_{II}$ is inhibited by infusion of Saralasin (91). Nevertheless, in spite of these data against the idea that autoregulation of RBF and GFR involves renin or $A_{II}$, the concept of tubuloglomerular feedback control of GFR is still viable. By perfusing Ringer's solution through the loop of Henle, Schnermann and co-workers have found that above a certain perfusion rate, stepwise increases in the rate of fluid delivery resulted in proportional decreases in the pressure within the capillaries of the corresponding glomeruli and (presumably) decreases in the single-nephron filtration rates (92). Perfusion below this rate, however, did not change the glomerular capillary pressure. Direct determinations of single-nephron GFR consistent with a feedback mechanism were also reported in subsequent studies in rats and dogs (93–95). In one of these, Wright & Schnermann (95) found that when inhibitors of distal NaCl transport such as furosemide, triflocin, or cyanide were added to the perfusing medium, single-nephron GFR responses to increases of perfusion rate in the loop of Henle were abolished. These investigators suggested that increased tubular transport of an electrolyte species in the distal nephron can be implicated as the link between rises in the rate of fluid movement through the loop of Henle and corresponding decreases in the single-nephron GFR (95). The renin-angiotensin system was not identified as the mediator of these responses, however. Other data indicate that tubuloglomerular feedback can be influenced by extracellular fluid volume expansion (96).

## THE RENIN-ANGIOTENSIN SYSTEM AND SODIUM IN HYPERTENSION

The incidence of hypertension in the adult population of the United States is approximately 15% (97). The public health consequences of this are obvious, in light of the data from the Framingham and other studies, which indicate that an elevated blood pressure (either systolic or diastolic) is a major risk factor for the development of stroke or heart attack (98). Hypertension may also lead to the development of congestive heart failure and kidney failure. Secondary causes of an elevated blood pressure (e.g. renal or adrenal diseases) are identified in less than 10% of the hypertensive population. The 90% of hypertensive patients for whom secondary causes are not found are defined as having essential hypertension, the cause of which is unknown. About 70% of patients with hypertension, including children, have a positive family history of high blood pressure (99, 100), suggesting the possibility of a genetic predisposition.

### *Renovascular Hypertension*

Hypertension due to renal artery stenosis is the most common surgically remediable form of hypertension (101). As such, it is the subject of intense interest for both clinicians and pathophysiologists. The surgical procedures to cure renovascular hypertension are not devoid of risk (102), and the cure rates currently achieved by surgery are low (101). For these reasons it is anticipated that a more complete understanding of the mechanisms responsible for the elevated blood pressure in this type of secondary hypertension may lead to more refined criteria by which patients are diagnosed and selected for surgery.

Since the classic experiment of Goldblatt, in which he produced sustained elevation of blood pressure by clamping the renal artery in dogs (2), it has been commonly accepted that abnormalities of the renin-angiotensin system are involved in the pathogenesis of renovascular hypertension. To facilitate a better understanding of interactions between the renin-angiotensin system and sodium in this disorder, experimental data are first reviewed and then clinical analogues of the experimental models of renovascular hypertension are examined.

EXPERIMENTAL DATA Two different experimental models of renovascular (Goldblatt) hypertension are commonly studied. In the one-kidney model, a silver clip is placed on one renal artery in order to reduce its blood flow, and the contralateral kidney is removed. In the two-kidney model, the renal artery of one kidney is clipped and the contralateral kidney is left intact. With these procedures, one-kidney or two-kidney Goldblatt hypertension can be easily induced in rabbits, rats, sheep, and dogs (103–109). In both models, removal of the clip on the renal artery is followed by a return of the blood pressure to normal, even after hypertension has been present for a long time (103, 104, 110–112). If, instead of removing the clip, one removes the kidney distal to the clip, blood pressure is also restored immediately to normal in two-kidney Goldblatt hypertension (103, 110, 113). In rats with one-kidney Goldblatt hypertension, removal of the remaining clipped kidney within 1–6 days after the clip has been placed is followed by a return of blood pressure to normal, but then there is a gradual increase in the level of the pressure similar to that observed in normal rats after total nephrectomy (113). However, if the solitary clipped kidney is removed after 4 weeks, blood pressure remains elevated (103, 110, 113).

In both experimental models of renovascular hypertension, clipping of the renal artery produces a reduction of renal blood flow in the affected kidney and increased secretion of renin by the juxtaglomerular cells (7, 104). In the two-kidney model (i.e. with the contralateral kidney intact), renin secretion and peripheral plasma renin activity remain elevated. In contrast, in the one-kidney model, plasma renin activity initially rises, then returns to normal or subnormal levels within a few days after the renal artery has been clipped. Therefore, the two-kidney form of Goldblatt hypertension has been accepted as the classical model of renin-dependent hypertension, and the one-kidney form has been considered to be a form of hypertension that did not depend on renin for maintenance of the elevated pressure. Why blood pressure returned to normal when one removed the clip from the renal artery of the single kidney in the latter model was unexplained.

Changes in sodium balance also occur during the development of experimental Goldblatt hypertension (104). Urinary sodium excretion falls acutely in response to constriction of a renal artery. This is secondary, in part, to increased renin secretion that generates increased circulating levels of $A_{II}$, which stimulates aldosterone production by the adrenals. Aldosterone promotes sodium reabsorption by the distal renal tubules (4). The reduction of renal blood flow distal to the renal artery stenosis, which may be associated with a reduced glomerular filtration rate, also enhances sodium reabsorption (114). It appears, therefore, that the acute rise in blood pressure that follows experimental renal artery constriction is associated not only with increased circulating $A_{II}$, but also with sodium retention by the kidney. However, investigators were unable to prevent the acute development of elevated blood pressure or to correct it completely in either model of Goldblatt hypertension by dietary sodium deprivation (115–117).

Later in the course of the development of renovascular hypertension, differences in the state of sodium balance between the one- and two-kidney models have been uncovered. Tobian found that total exchangeable sodium (a measure of sodium in the extracellular space) was normal in the two-kidney model, but was increased significantly above normal in rats with one-kidney Goldblatt hypertension (118). It appears that when the arterial pressure rises in the animals with two-kidney renovascular hypertension, increased sodium retention by the stenotic kidney is counterbalanced by increased sodium excretion by the kidney contralateral to the clip [possibly a "pressure natriuresis" (119, 120)]. Because the sodium balance remains unchanged in this situation, renin secretion by the stenotic kidney continues at inappropriately high rates.

In the one-kidney model, the total renal mass is reduced. Sodium retention by the single clipped kidney expands extracellular fluid volume and sodium stores until the systemic arterial pressure rises to levels where the impaired excretory capacity of the stenotic kidney is overwhelmed. At this point, a pressure-induced diuresis of sodium and water ensues, establishing a new equilibrium where sodium excretion equals intake (104). In the face of a positive sodium balance and an increased pressure, renin secretion by the kidneys declines and plasma renin activity returns to normal levels.

Because of the differences in plasma renin activity in one- and two-kidney Goldblatt hypertension, investigators attempted to define the pathogenetic role of the renin-angiotensin system in these models by actively or passively immunizing animals to different components of the system. The experiments yielded conflicting and inconclusive results (121–125). Important new information however, was provided by experiments using two pharmacological blockers, Saralasin and CEI (126, 127). Administration of the blockers in the two-kidney model during any phase of the hypertension produced a rapid fall in blood pressure. In the one-kidney model, the blockers reduced blood pressure in the very early phase, but did not lower blood pressure in the established phase of hypertension (124, 128, 129). These experiments pointed to a significant role for the renin-angiotensin system in initiating hypertension in both types of Goldblatt hypertension. However, a role for the system in sustaining hypertension appeared to be present only in the two-kidney model.

More recent experiments by Gavras et al (49, 130) indicate that both sodium and the renin-angiotensin system co-participate in the maintenance of established hypertension in both the one- and two-kidney models of Goldblatt hypertension. Although one-kidney animals fail to have a hypotensive response to infusions of the angiotensin inhibitors when they are replete with sodium, arterial pressure falls promptly upon administration of similar doses of inhibitors after the animals have been subjected to chronic sodium deprivation or acute sodium depletion (49). A similar pattern is also observed in two-kidney rats. Although $A_{II}$ inhibitors reduce the arterial pressure of sodium-replete two-kidney Goldblatt rats after 4–6 weeks of hypertension, they no longer lower blood pressure at 14–16 weeks. If at this time the animals are depleted of sodium, they again respond to the inhibitors with a fall in blood pressure (130). Presumably, the chronic hypertension of the two-kidney animals eventually produces subtle vascular and parenchymal damage in the contralateral unclipped kidney, which impedes its capacity to excrete sodium, so that sodium retention results (130). In this situation, depletion of sodium by diet or diuretics unmasks a latent role of $A_{II}$ to maintain the hypertension. Sodium depletion does not lower the blood pressure by itself, however.

These data, which suggest that there is reciprocity between renin dependency and sodium dependency in the maintenance of various forms of experimental renovascular hypertension, may also provide insights into renovascular and other forms of human hypertension (see below).

CLINICAL RENOVASCULAR HYPERTENSION Stenosis of a main renal artery or one of its branches is a well-documented cause of human hypertension occurring in about 2–5% of the entire hypertensive population (101, 131). The pathological lesions associated with renovascular human hypertension are numerous. Fibromuscular hyperplasia of the vascular wall and atherosclerotic plaques, which partially occlude the orifice or lumen of the renal artery, are the most frequent lesions in young women and older males, respectively (101). The presence, location, and extent of such lesions can be demonstrated by aortography and selective renal arteriography (132). Whether a given renal artery lesion is the cause of the hypertension in a given patient is not always clear, however. Lesions of equal anatomic severity are frequently observed in patients with normal or high blood pressure (133, 134). In many patients with renal artery stenosis, successful surgical revascularization or uninephrectomy has failed to reduce the elevated pressures to normal (101, 135, 136). Renovascular surgery, particularly in older patients with atherosclerosis, has a mortality rate on the order of 6–10%, and considerable morbidity (101, 137).

For these reasons, the renin-angiotensin system has been investigated in patients to determine whether alterations of its components might predict when revascularization of an ischemic kidney in a hypertensive patient is likely to be followed by a restoration of normotension.

Early studies revealed that peripheral plasma renin activity varied widely in patients with hypertension cured by revascularization of the kidney or nephrectomy. In a minority of patients with hypertension, usually of very recent onset of the severe or malignant variety, peripheral plasma renin activity was increased (138, 139). In the majority with sustained nonmalignant hypertension, peripheral renin levels were normal (138, 139). In attempts to predict the outcome of surgery, measurements were made of renin activity in blood obtained by selective catheterization of the renal veins, and the ratio of renin activities in the two sides was taken as an index of asymmetrical renin release (140–143). Several maneuvers were also devised to enhance differences in renin secretion between the two kidneys; these included stimulation of renin secretion by upright posture (144), administration of vasodilators (145), and sodium depletion (146). Claims were advanced that a ratio of renal vein plasma renin activity concentrations between the two sides of 1.5 or greater indicated curability in about 80% of patients (147, 148).

However, the concentration of renin in samples of renal venous blood is influenced by factors other than renin secretion by the kidney (such as a reduced rate of renal blood flow, the position of the sampling catheter, admixture of venous blood from other veins, etc). An analysis based exclusively upon renal vein plasma activity ratios also ignores the absolute baseline renin levels. Accordingly, Vaughan et al (139) suggested that three indicators, in combination, might have a greater predictive value. These were (*a*) a value for peripheral renin activity that was increased in relation to urinary 24-hr sodium excretion; (*b*) suppression of renin secretion by the contralateral kidney, i.e. a value of renin activity in renal venous blood from the nonstenotic kidney equal to or lower than that in peripheral blood or in blood of the inferior vena cava below the renal vein; and (*c*) renin levels in the renal vein blood of the stenotic kidney that exceeded values in the arterial blood or in blood from the lower inferior vena cava. These investigators found that a ratio of (V–A)/A of >0.48 (where V= renal vein renin activity and A= arterial renin activity) on one side was indicative of an ipsilateral functional renal artery stenosis. By these criteria, all 13 patients with a (V–A)/A ratio of >0.48 benefited by renal revascularization or by nephrectomy. However, due to the sampling errors mentioned and to difficulties in standardizing renin assays, these findings have not yet been confirmed in other laboratories.

Recently, administration of the competitive antagonist of $A_{II}$ (Saralasin) or of CEI to patients with renal artery stenosis has been proposed as a test to predict when the renin-angiotensin system is involved in the production of an elevated blood pressure (65, 149, 150). Since lowering of blood pressure during administration is an indication that hypertension is due to the increased renin secretion by the stenotic kidney, this finding might conceivably be used to predict surgical curability in patients with renovascular hypertension. Even though blood pressure reductions have been produced by administration of $A_{II}$ blockers to such patients, it is important to realize that normalization of blood pressure indicates only that the hypertension is renin dependent. It does not indicate that all of the increased renin secretion is arising from the kidney with the lesion that has been visualized by arteriography. As has been discussed previously, the degree to which the maintenance of normotension or hypertension is mediated by the renin-angiotensin system depends critically on the concomitant state of sodium balance. The optimal state of sodium balance, in which this $A_{II}$ blockade test can be applied and standardized in a large number of patients to obtain the maximal amount of information that might have a predictable value, is not yet known. Many investigators are currently addressing this question.

Finally, it must be acknowledged that the only proof that an occlusive lesion of the renal vasculature is producing hypertension is the normalization of the blood pressure after surgical relief of the renal arterial obstruction or after nephrectomy. The full cause of renovascular hypertension is unknown. Although the focus of this review is upon the renin-angiotensin system and the sodium ion, it is known that within the kidney there exist other vasoactive substances, such as the prostaglandins and the kinins. These intrarenal hormones also have potent effects on blood pressure, intrarenal circulation, and sodium homeostasis (58, 59, 63). Indeed, increased PGE-like activity levels and decreased kallikrein have been measured in the renal venous blood and urine, respectively, from ischemic kidneys (58, 151). Uncertainty concerning the full, and possibly protective, role of these renal vasodepressor systems is another reason surgical revascularization of the kidney is preferable to nephrectomy in the treatment of human renovascular hypertension.

### *Hypertension Associated with Mineralocorticoid Excess*

HYPERALDOSTERONISM In 1954, Conn first described a curable form of hypertension due to autonomous secretion of aldosterone by one or more adenomas of the adrenal gland (152). The syndrome, termed primary hyperaldosteronism, was characterized generally by mild hypertension, marked hypokalemia and alkalosis, and normal or elevated serum concentrations of sodium and chloride. Aldosterone urinary excretion or adrenal secretion rates were increased. The elevated blood pressure and electrolyte abnormalities were, in most cases, restored to normal by surgical removal of the tumor. The incidence of this disorder has been debated; current evidence suggests that primary hyperaldosteronism occurs in about 1–2% of the hypertensive population (153, 154).

Primary hyperaldosteronism must be distinguished from various forms of secondary hyperaldosteronism. In patients with malignant or advanced essential hypertension or renovascular hypertension, increased aldosterone secretion and excretion may be secondary to excess production of renin by damaged kidneys, leading to increased plasma renin and $A_{II}$ concentrations, and consequent stimulation of aldosterone secretion (4). These patients, who usually have severe hypertension and hypokalemia, do not have adrenal tumors and their blood pressure is not improved by adrenalectomy. In primary hyperaldosteronism, on the other hand, autonomous and continuous aldosterone excess leads to accumulation of sodium and water, expansion of body sodium stores and of effective intravascular blood volume, and suppression of renal renin secretion. Thus, the combination of subnormal plasma renin activity (PRA) with elevated aldosterone production was accepted as the criteria for diagnosis of primary hyperaldosteronism (155), while increased PRA along with increased aldosterone was diagnostic of secondary oversecretion of the hormone.

A sizable percentage of patients (up to 50% in some series) who exhibited hypersecretion of aldosterone and suppressed PRA were discovered at surgery not to have the expected small adrenal cortical adenoma(s) producing aldosterone, but rather, either a diffuse nodular adrenal hyperplasia or no demonstrable pathology at all. In most cases, total or partial adrenalectomy failed to cure or benefit the hypertension in this situation (156–159). The etiology of this condition, which has been given several designations including idiopathic hyperaldosteronism (160) or pseudoprimary hyperaldosteronism (159) is unknown. Some workers postulate that the adrenal is stimulated by a nonrenal stimulus. Others speculate that in certain patients the condition may represent a tertiary form of aldosteronism, in which an initial continuous stimulation of the adrenals by excess renin-$A_{II}$ leads eventually to the development of either nodular adrenal hyperplasia or multiple autonomously secreting adenomas with consequent suppression of plasma renin activity.

Since the probability that hypertension will be alleviated by surgery in idiopathic hyperaldosteronism is low, accurate preoperative differentiation from hyperaldosteronism due to an adrenal adenoma is important. Unfortunately, there are at present no definitive diagnostic criteria. In general, the biochemical and electrolyte abnormalities tend to be milder in patients with the idiopathic form in those with adenomas, i.e. (*a*) the hypokalemia and alkalosis are lesser in magnitude, (*b*) the aldosterone secretion rate is elevated to a lesser degree in the idiopathic form and the degree to which secretion of the hormone can be suppressed is greater, and (*c*) plasma renin activity is not so low and can be stimulated to a greater extent by sodium depletion in the idiopathic form than in hyperaldosteronism due to adenomas. However, in all of these parameters there is considerable overlap between the two conditions. Even normokalemia does not completely exclude the presence of an adrenal adenoma (161), since lowering of the serum potassium concentration by excessive aldosterone secretion may be intermittent or dependent on the state of sodium balance (162). These uncertainties have led some workers to apply a computer-assisted statistical analysis to the preoperative biochemical data to facilitate more accurate diagnosis of the condition (156). Early claims (163) that the magnitude of the response of blood pressure and of the electrolyte abnormalities to spironolactone treatment would aid in making the distinction are doubtful, since both forms of primary hyperaldosteronism (156) as well as low-renin essential hypertension with and without mineralocorticoid excess respond equally well to spironolactone (164, 165).

Other approaches to distinguishing between the two conditions have focused on the attempt to localize an adenoma by measuring aldosterone concentrations in blood obtained from both adrenal veins by femoral catheterization (166) or from the left adrenal vein and the inferior vena cava (which is technically easier) (167). The success rate of these procedures, however, is not optimal, even in experienced hands, because of problems such as pulsatile adrenal steroid secretion, admixture of venous blood from other organs, tumors too small to be visualized, adrenal infarction after catheterization, etc. Visualization of adrenal adenomas by external imaging with a scintillation camera after the intravenous administration of $^{131}$I-19-iodocholesterol (168) is another promising approach that has not been widely applied because of the high radiation dose to the patient with current scintigraphic equipment.

Recently, however, it has been reported that aldosterone secretion by adrenal adenomas is not influenced by changes in patient posture (renin stimulation), but has a circadian rhythm; plasma aldosterone levels are highest in the morning and decline during the day. In contrast, aldosterone secretion in bilateral adrenal hyperplasia shows no circadian rhythm and plasma aldosterone levels increase when the patient assumes an upright position. Successful preoperative differentiation of primary and idiopathic hyperaldosteronism has been achieved by determining these patterns (169, 170).

Two other rare variants of primary hyperaldosteronism with suppressed PRA have also been described. In one type, the administration of desoxycorticosterone suppresses aldosterone secretion and excretion down to the normal range (171). In the other, a familial disorder, the administration of glucocorticoids suppresses aldosterone oversecretion, corrects hypokalemia, and restores blood pressure to normal (172, 173).

OTHER ADRENAL STEROIDS Hypertensive patients with Cushing's syndrome frequently present with hypokalemia and alkalosis; in these patients adrenal secretion of glucocorticoids and abnormal steroid precursors is excessive, regardless of whether the syndrome results from adrenal hyperplasia, tumor, or ectopic ACTH production. Patients with Cushing's syndrome generally have normal aldosterone secretion rates, increased renin substrate, and normal PRA (174, 175); diagnosis usually presents little problem.

Excessive circulating levels of mineralocorticoids other than aldosterone have been implicated in the pathogenesis of hypertension in additional patients with elevated blood pressure who manifest hypokalemia and alkalosis. Although PRA is suppressed in these rare disorders, the secretion rates of aldosterone are normal or subnormal. These disorders include two rare forms of congenital adrenal hyperplasia that are associated with hypertension. In 17-$\alpha$-hydroxylase deficiency there is primary amenorrhea or male pseudohermaphroditism (176); in the 11 $\beta$-hydroxylase deficiency there is early virilization (177). In both, urinary 17-ketosteroid excretion is elevated, and there is increased adrenal production of desoxycorticosterone (DOC) and other steroid biosynthetic precursors of aldosterone (178). Patients with hypertension associated with isolated excess of desoxycorticosterone (179) or 18-hydroxy-desoxycorticosterone (180) or of other mineralocorticoids (181, 182) have also been described. They have suppressed PRA and varying degrees of hypokalemia. Diagnosis is achieved by measurements of the appropriate steroids; treatment with spironolactone is generally effective in correcting the electrolyte disturbances and in lowering the blood pressure.

Finally, a rare familial form of hypertension has been described by Liddle (183) in which hypertension is associated with hypokalemic alkalosis, suppressed PRA, and markedly reduced adrenal secretion of aldosterone. Although the adrenal production of mineralocorticoids is reduced, the kidneys have an abnormal tendency to retain sodium. Patients with the syndrome are unresponsive to spironolactone, but hypokalemic alkalosis and the elevated blood pressure are corrected by the diuretic triampterene.

CAUSE OF THE HYPERTENSION In Liddle's syndrome and in the other hypertensive disorders produced by excessive adrenal production of aldosterone or other mineralocorticoids, plasma renin activity is suppressed at rest and there is a subnormal response to procedures that tend to stimulate renin secretion by the kidneys, such as sodium depletion (178, 183). In addition, there is evidence for increased body sodium and increased plasma and extracellular fluid volumes (152, 178, 183). These features, plus the frequently beneficial effects of treatment with spironolactone (163, 184) or other diuretics suggest that the hypertension results primarily from renal retention of salt and water. It would appear that the renin-angiotensin system plays only a minor role, if any, in the maintenance of an elevated blood pressure.

More information is needed, however, concerning genetic influences on the development of hypertension during induced sodium retention. Dahl and his co-workers, using selective breeding, were able to develop a strain of rats that rapidly developed hypertension when the sodium content of the diet was increased, and another strain that was resistant to the development of hypertension when subjected to the same dietary stress (185). In these rats, genetic predisposition toward hypertension or resistance to hypertension during salt loading was transmitted to recipient animals by renal transplantation (186). That genetic factors may be operative in the production of human hypertension in response to sodium retention is suggested by a report of Biglieri et al (160). These workers found that 60% of a large series of patients with primary hyperaldosteronism were rendered normotensive by adrenal surgery; the others remained mildly hypertensive or again developed an elevated blood pressure months to years later, which responded to treatment with diuretics and other antihypertensive agents.

### *Essential Hypertension*

For many years investigators have postulated that essential hypertension was the manifestation of a multifaceted derangement of the homeostatic mechanisms that control blood pressure. Most have felt that from the large number of patients with essential hypertension, an increasing number of subsets of patients will be identified in whom specific pathogenetic mechanisms operate to increase the arterial blood pressure. During the last decade, classification of patients with essential hypertension according to the level of PRA has been advocated (4). Patients were divided into three subgroups: normal, low renin, and high renin. It was suggested that this classification had not only diagnostic but also prognostic and therapeutic value.

In the most extensive studies, by Laragh and his associates (187), patients were grouped according to their plasma renin levels; these were regarded as low, normal, or high when related to a measure that reflected the sodium balance of the patient. Values of PRA in hypertensives were compared to those in normals by a nomogram relating PRA and the 24-hr urinary sodium excretion rate. Although the following discussion is based on such a classification, recent data has indicated that renin values decline with age in both normotensives and hypertensives (188), and that renin values at all ages are lower in blacks than in whites (189–191). It is quite possible that current classifications of patients according to PRA levels that are not factored for age or race will require additional modification. Furthermore, the question of whether a 24-hr urinary sodium excretion rate is a sufficiently sensitive indicator of the steady-state total-body sodium stores for use in defining normality of PRA has not yet been adequately answered.

NORMAL RENIN HYPERTENSION The majority of patients with essential hypertension, about 57%, exhibt PRA levels within the normal range (187). Most of these patients also have normal aldosterone secretion and normal renin-aldosterone relationships over a wide range of altered sodium balance (187, 191, 192). Whether the renin-angiotensin system plays a pathogenetic role in normal-renin essential hypertension is unclear. Some investigators believe that it does not, particularly in view of the therapeutic efficacy of diuretic drugs and of antihypertensive agents that inhibit the sympathetic nervous system, such as guanethedine. Others have speculated that transient elevation of renin-$A_{II}$ may have stimulated aldosterone secretion in early stages of the disease, leading to retention of sodium and increased vascular sensitivity to pressor effects of $A_{II}$ (4, 55). Thereafter, lower levels of $A_{II}$ could maintain high blood pressure (44), while circulating renin and aldosterone concentrations returned to normal. Since elevation of blood pressure should normally lead to natriuresis and return of pressure to normal (119, 120), it is also possible that slight impairment of sodium excretion by the kidney occurs, which leads to an accumulation of sodium in the extracellular space and in the tissues; in this situation, renin levels would be suppressed. By such reasoning, the normal plasma renin activity levels found in normal-renin hypertension may actually be high in regard to the postulated increase in tissue sodium stores.

The administration of blockers of $A_{II}$ (Saralasin) to sodium-replete, normal-renin hypertensives is not followed by a reduction in arterial blood pressure. However, if the patients are sodium depleted by diuretics, administration of either agent results in a lowering of the increased blood pressure (65, 149, 193). This suggests participation of the renin-angiotensin system in the maintenance of an elevated pressure in these particular circumstances. Similarly, the antihypertensive response of normal-renin, sodium-replete hypertensives to drugs that suppress renin secretion by the kidneys is inconsistent (194). Diuretics are still the most widely used antihypertensive agents. However, because the rise in renin secretion induced by diuretics in hypertensives has been suspected of participating in vascular damage [i.e. the development of myocardial infarction (195) and malignant hypertension (196)], some investigators have advocated the use of renin-suppressing drugs such as propranolol in normal renin hypertension either as drugs of first choice (194) or in combination with other agents.

LOW-RENIN HYPERTENSION The incidence of suppressed PRA (i.e. renin levels that are low in relationship to the 24-hr urinary sodium excretion or hyporesponsive to physiological stimuli) ranges in different studies from 25 to 47% of essential hypertensives (187–192). The variation is probably due to differences in the renin assays used, age and race factors mentioned previously, differences in the indexes used to reflect sodium balance, and different methods used to test the responsiveness of the renin-secretory apparatus. The latter include a low-salt diet, the response to upright posture, an acute challenge with furosemide (154), and the administration of vasodilators such as diazoxide (197).

The aldosterone secretory patterns associated with suppression of plasma renin in low-renin hypertension vary. Patients with suppression of both renin and aldosterone secretion have been observed, along with others in whom renin was low and aldosterone secretion was normal (198). In the latter group, both hormones rose and fell together in response to changes in sodium balance (198). The different hormonal patterns in these patients have led most investigators to believe that the classification of low-renin essential hypertension is a grouping that contains subsets of patients in which the suppression of renin secretion results from different causes.

A variety of theories have been advanced (199). It has been mentioned previously that both PRA levels and aldosterone secretion rates decline with age. Conceivably, in some patients with essential hypertension, evolution from the normal-renin to the low-renin status might occur as age advances. Thus for a certain percentage of hypertensives, low-renin hypertension might represent a later state in the natural history rather than a separate entity.

Blood and extracellular fluid volumes (200, 201) and total exchangeable sodium (202) were reported to be increased in low-renin hypertensives above values obtained in normal-renin hypertensives. These data, plus reports that treatment with diuretics (both thiazides and spironolactone) was particularly efficacious in lowering blood pressure in the low-renin group (164), has led to the theory that body sodium stores and extracellular fluid volume are increased in the disorder as a result of abnormal sodium retention by the kidneys. Whereas some workers suggest that extracellular fluid volume expansion in low-renin essential hypertension results from secretion of mineralocorticoids other than aldosterone by the adrenal cortex, others postulate that there is a primary disorder of kidney function (199).

Increased urinary excretion or adrenal secretion of mineralocorticoids such as DOC, 18-hydroxy-DOC, 16$\alpha$-18-dihydroxy-DOC, and 16$\beta$-hydroxy-dehydroepiandrosterone have been measured in small numbers of patients with low-renin hypertension who were not hypokalemic (178–182). In other studies, treatment with the mineralocorticoid antagonist, spironolactone (164) or aminoglutethimide, an inhibitor of adrenal steroidogenesis (202), decreased blood pressure and returned suppressed PRA to normal. One group performed bilateral adrenal exploration in seven low-renin patients who had normal aldosterone secretion rates; adenomas were present in four patients, and adenomatous hyperplasia in three (203). Taken together, these observations suggest that at least in some patients, abnormal adrenal steroidogenesis may be responsible for the hypertension.

Other evidence, however, can be found to support the hypothesis that the condition results not from a primary adrenal disorder, but from abnormalities of renal function that result in retention of salt and water. In support of the idea that adrenal pathology need not imply a causal role in the production of increased blood pressure, an autopsy study revealed that 6.9% of unselected hypertensives and 1.9% of unselected normotensives have adenomas, nodules, or hyperplasia of the adrenals (204). In two studies, spironolactone and a thiazide diuretic were equally effective in lowering the blood pressure of low-renin and normal-renin patients with essential hypertension (165, 205). In another, 80% of unselected hypertensives had a beneficial response to thiazides alone (206). These data, plus the experimental studies and epidemiological studies that relate salt ingestion to the incidence of hypertension (207, 208), support the hypothesis that many low-renin hypertensives may have increased retention of sodium and water by the kidney and suppression of renin secretion as a consequence of a slight expansion of the blood and extracellular fluid volumes. Observations counter to this idea, such as reports of normal plasma and extracelluar fluid volumes (200) or exchangeable sodium (209, 210) in low-renin patients, may be explained if low-renin hypertension is indeed a grouping of heterogeneous patients.

Despite current uncertainty concerning etiology, low-renin hypertension may have clinically important characteristics. In a retrospective study, Brunner et al found that the incidence of stroke and heart attack was lower in low-renin hypertensives than in the normal or high-renin group (192). This observation has been confirmed by some groups, but not by others (199, 211, 212). The postulate that the level of renin activity may be an additional risk factor for the development of stroke or myocardial infarction is consistent with known vasculotoxic effects of angiotensin on the cerebral and myocardial vasculature (213). At present, however, the quantitative significance of an additional risk implied by a given level of PRA is uncertain. Therapeutically, however, it has been generally observed that the blood pressure of low-renin essential hypertensives is benefited by diuretic therapy, which successfully reduces body sodium stores (165). Blood pressure in low-renin hypertension is not generally benefited by treatment with renin-suppressing drugs such as propranolol; in fact, propranolol administration to such patients may occasionally precipitate additional fluid retention and the development of congestive heart failure (194).

HIGH-RENIN HYPERTENSION About 16% of patients with essential hypertension have been reported to have plasma-renin activities that are elevated above normal in relation to 24-hr urinary sodium excretion (4, 187, 191, 192). Such patients generally have higher blood pressures than the other subgroups (192). They usually have a mild degree of hypokalemic alkalosis and commensurately increased aldosterone secretion rates. However, in some patients with hypokalemia, aldosterone secretion rates are low in relationship to the increased renin, probably reflecting the influence of a low serum potassium to retard aldosterone secretion by the adrenal (66). Retinopathy and evidence of mild impairment of kidney function are not uncommon. High-renin hypertension in some patients may precede the development of the malignant hypertension syndrome.

Studies with peripheral $A_{II}$ blockade and CEI indicate that $A_{II}$ is directly involved in the pathogenesis of high-renin hypertension. During infusion of these substances, blood pressure falls promptly towards or to normal levels (65, 149, 150, 193). Renin activity in plasma rises during $A_{II}$ blockade, suggesting interruption of a normal feedback effect of $A_{II}$ to suppress renin output. Although the role of the renin-angiotensin system seems to be dominant, the state of sodium balance also influences the level of increased arterial pressure in high-renin essential hypertension. Sodium depletion enhances the hypotensive effect of $A_{II}$ blockade, while infusion of saline may prevent or abolish it (193). The demonstration that a patient is in the high-renin subgroup may have prognostic and therapeutic implications. In the study of Brunner et al (192) the incidence of heart attack and stroke was highest in this group (which also had the highest diastolic blood pressure). Antihypertensive treatment with renin-suppressing drugs such as propranolol has been most efficacious in the high-renin group (194). Restoration of normotension usually occurs without the side effect of sodium retention by the kidney.

### *Malignant Hypertension*

The syndrome of malignant hypertension may develop in patients in the course of essential hypertension, renovascular hypertension, and all forms of secondary hypertension, with the possible exception of coarctation of the aorta. The syndrome is characterized pathologically by a necrotizing vasculitis involving many tissues, particularly the kidneys (213, 214). In humans it is characterized by high levels of diastolic blood pressure, retinopathy with hemorrhages, exudates, and often papilledema and encephalopathy; the hallmark is progressive deterioration of renal function. There may also be weight loss, polyuria, and evidence of hypovolemia. A similar picture occurs in animals with experimental malignant hypertension.

Most patients with malignant hypertension have very high levels of plasma renin activity, high circulating aldosterone, and hypokalemia and alkalosis despite impairment of renal function (4). It was the finding of oversecretion of aldosterone along with evidence of renal damage in such patients that led to the discovery that $A_{II}$ increased aldosterone secretion and excretion in man (215). The administration of angiotensin inhibitors (Saralasin and CEI) to patients with malignant hypertension has been associated with marked reductions in arterial blood pressure, diuresis in some cases, and improvement in myocardial function (193). In fact, such drug treatment in selected cases may be faster and more effective than propranolol, which may also depress cardiac function. The accumulated data support the proposal (4) that in malignant hypertension an abnormal situation occurs whereby renal damage causes increased renin and aldosterone output, which in turn produces sodium retention. Because of renal damage, possibly induced by excessive hormone levels, the normal sodium feedback inhibition of renin secretion is defective and a vicious cycle results whereby inappropriate renin secretion persists and perpetuates vascular damage.

The idea that increased circulating $A_{II}$ is vasculotoxic is supported by a variety of experimental observations. Winternitz et al in 1940 (216) induced necrotizing vascular lesions in dogs by bilateral renal artery ligation and by administration of kidney extracts. Similar lesions were induced by administration of semipurified hog renin to nephrectomized rats and later in the same experimental model by small doses of $A_{II}$, and in animals with intact kidneys by large doses of the pressor octapeptide (217, 218). In several animal species, infusions of $A_{II}$ produce constrictions of the arterioles that alternate with areas of segmental dilatation, which are the sites of increased permeability of the vessel wall (219, 220). Angiotensin II also increases vascular wall permeability (221) by causing contraction of endothelial cells and widening of intraendothelial junctions (222). This enhances the deposition of plasma constituents in the vascular wall, so-called plasmatic vasculosis (223). Other vasculotoxic effects of $A_{II}$ that have been described in experimental animals include development of acute renal failure (224) and of focal microscopic areas of myocardial infarction (225).

Masson et al (226) reported that in rats injections of renin or aldosterone alone did not produce vascular lesions, but that injections of both hormones induced rapidly fatal vasculitis. In contrast to the suggestion that both hormones are necessary for production of vascular damage, several cases of malignant hypertension with necrotizing vasculitis have been reported in patients with normal (227) or suppressed levels of plasma renin activity (228–230). More recently, Gavras et al reported that the vascular lesions of malignant hypertension were produced experimentally in rats treated with desoxycorticosterone acetate (DOCA) and salt in which renin activity was suppressed (231). Administration of a competitive antagonist of $A_{II}$ to these animals failed to lower blood pressure, additional evidence that $A_{II}$-mediated vasoconstriction was not necessary for the production of the lesions.

In the DOCA-salt animals, and in experimental renovascular (renin-dependent) hypertensive rats, the onset of the malignant phase of the hypertensive disease was heralded by paroxysms of natriuresis associated with weight loss and hemoconcentration (231, 232). It has been postulated that in these models, loss of salt and water with associated hemoconcentration and increased blood viscosity results in diminished perfusion, damage to subendothelial arteriolar tissue, and (possibly) to intravascular coagulation and deposition of fibrinoid substance in the vascular wall (233, 234). Although these data demonstrate that $A_{II}$ is not a prerequisite for the development of vascular damage, more work is required to document the sequence of events that produces the vascular damage of malignant hypertension. Despite these uncertainties, effective restoration of normotension results in a restoration of renin and aldosterone levels to normal (213, 235) improvement of renal function and the retinal damage (236) and a healing of the arteriolar necrotizing lesions (237, 238).

## THE RENIN-ANGIOTENSIN SYSTEM IN ACUTE AND CHRONIC RENAL FAILURE

Since the renin-angiotensin system is involved to a major extent in the pathogenesis of malignant hypertension, characterized by renal vascular damage and impaired function, investigators have also attempted to define its role in acute renal failure and hypertension associated with chronic renal failure.

### *Acute Renal Failure*

Despite impressive advances in the understanding of normal renal function in the last 30 years, the pathophysiology of acute renal failure (ARF) is still not understood. Four mechanisms have been offered to explain the observed decrease in the rate of glomerular filtration in ARF: (*a*) vasospasm of the afferent renal arterioles with a drop in glomerular filtration pressure, (*b*) tubular damage with a back-leak of filtrate, (*c*) tubular obstruction by cellular debris or interstitial edema, and (*d*) depression of the permeability of the glomerular structures through which filtration occurs. Due to the inherent difficulty of performing studies in patients with ARF, most work directed at underlying mechanisms has been performed in animals.

Goormaghtigh in 1945 reported hypertrophy of cells of the juxtaglomerular apparatus in kidneys of patients who died with ARF secondary to trauma (239). Subsequently, in patients with ARF due to a wide variety of causes, such as acute tubular necrosis (240), nephrotoxins (241), cirrhosis with spontaneous renal failure (242), and scleroderma (243), marked reductions of mean RBF and flow in outer cortical regions of the kidney have been reported by investigators who used inert gas washout techniques to measure blood flow per unit volume of tissue. In these and most clinical forms of ARF, very high peripheral PRA and elevated $A_{II}$ blood levels were found (244–246). In addition, peripheral renin activity was increased (247, 248) and cortical blood flow was reduced in several experimental models of ARF (249–251). These observations, plus the report by Gavras et al that very high doses of $A_{II}$ induced oliguric renal failure in rabbits (224), have raised the question whether the renin-angiotensin system might be involved in the pathogenesis of some forms of ARF. Thurau and co-workers induced ischemic renal failure by renal artery occlusion in rats and found an inverse correlation between distal tubular sodium concentration and single-nephron GFR in the animals in the postischemic period (86, 252). The implication of these experiments was that derangement of a tubuloglomerular feedback mechanism that involved renin and angiotensin might be responsible for the decreased GFR in postischemic renal failure by producing vasoconstriction of the afferent arterioles.

More recent studies, however, have failed to provide conclusive data implicating renin-angiotensin in the production of oliguric renal failure. In postischemic renal failure (253–255) and in acute renal failure induced by intrarenal infusion of norepinephrine (256), oliguria persisted despite restoration of total renal blood flow to control levels. Furthermore, recent studies have shown that in contrast to previous reports (249, 257), renal blood flow was normal in uranyl nitrate-induced ARF at a time when GFR was markedly depressed (258).

Oken and his co-workers investigated the role of renin in ARF induced by glycerol or mercuric chloride. They found that rats pretreated with a high sodium intake to lower peripheral and kidney renin had a lesser deterioration of kidney function and more rapid recovery after the experimental insult than did rats fed a lower sodium intake (259–261). The protection afforded by saline loading in these experiments was unrelated to urine volume and osmolality (259, 260, 262). Flamenbaum et al reported a similar reduction of the effect of $HgCl_2$ to induce ARF in rats in which plasma and kidney renin was depleted by potassium loading (263).

In contrast to the above studies, other workers who used immunological and pharmacological techniques to suppress activity of the renin-angiotensin system obtained discordant results in experimental ARF. Powell-Jackson et al demonstrated that rats passively immunized against $A_{II}$ were significantly protected from glycerol-induced ARF (264). Flamenbaum et al, on the other hand, were unable to affect the renal response to glycerol and to $HgCl_2$ in rats immunized against renin, despite depressions of the plasma renin activities comparable to that observed in sodium-loaded animals (265). A subsequent combined study by these last two groups showed that neither passive nor active immunization to $A_{II}$ significantly affected the degree of azotemia and the reduction of the GFR in rats poisoned with glycerol (266). Similarly, administration of a competitive antagonist to $A_{II}$ (Saralasin) or of CEI, did not improve renal function in glycerol-treated rats (267) or in rats with postischemic renal failure (253). Further work in this area is needed to clarify the role of renin-angiotensin in ARF. Techniques must be devised to indicate whether the inhibitors block not only peripheral angiotensin, but also angiotensin that may act locally within the kidney.

Despite current uncertainty concerning the participation of the renin-angiotensin system in ARF, the recent application of sophisticated micropuncture techniques to study experimental models of ARF is providing more definitive information concerning mechanisms responsible for the oliguria. In response to transient renal ischemia, reductions of capillary plasma flow were found to be associated with diminished GFR (268). Blantz obtained data consistent with reductions in glomerular permeability in rats with ARF from uranyl nitrate ingestion (258). Indirect evidence suggesting a reduction in glomerular permeability was also obtained in uranyl nitrate-treated dogs and in dogs with ARF secondary to intrarenal norepinephrine infusion. Scanning electron microscopy of the glomeruli in those two models showed abnormalities of the epithelial cells that support the glomerular basement membrane (256, 269). Other workers have obtained data indicative of excessive back-diffusion of ultrafiltrate in several models of ARF (254, 258, 269, 270). Finally, two groups have obtained evidence documenting an important role for intratubular obstruction in both the generation and maintenance of the oliguria following temporary renal artery obstruction (253, 255).

### *Hypertension Associated with Chronic Renal Failure*

High blood pressure frequently develops in patients with chronic renal failure. The mechanisms producing the elevated blood pressure are similar, regardless of the disease that produced chronic bilateral destruction of the renal parenchyma. Many patients with markedly reduced kidney function (even with uremia) never become hypertensive, however.

In a majority of patients with chronic renal failure, the accumulation of excessive amounts of salt and water appears to play an important role in the pathogenesis of their hypertension. Measurements of plasma volume and exchangeable sodium in patients with renal failure (271–273) showed that increases in these values were directly correlated with the level of arterial blood pressure. PRA was normal or subnormal (274, 275). Depletion of sodium and fluid by hemodialysis effectively restored normal blood pressure in this group of patients (275–277).

Experimental analogues of sodium-dependent hypertension in renal disease have been produced in animals. Ablation of two thirds to three fourths of the functional renal mass by subtotal nephrectomy in rats and dogs is followed by development of a form of hypertension that is aggravated by increased sodium intake (278–281). A positive sodium balance is associated with increases in extracellular fluid (ECF) and blood volumes and in the arterial blood pressure in subtotal nephrectomized dogs (104, 278–280). Cardiac output rises during the initial days following salt ingestion and then declines, and the increased blood pressure appears to result from an increased total peripheral vascular resistance (278, 282).

Measurements by Coleman et al in anephric humans maintained on hemodialysis revealed a similar pattern (283). In response to a positive sodium balance, cardiac output and blood pressure increased. Total peripheral resistance, initially normal, increased during sodium loading; this was followed by a return of the cardiac output to normal and maintenance of the elevated blood pressure. In studies of large numbers of patients with renal failure associated with hypertension, it was found that plasma and ECF volume were above normal; in the majority of patients, total peripheral resistance was increased (284, 285), while the cardiac index was either normal or increased (particularly in those with anemia).

In addition to this larger group, in whom blood pressure elevation appears to be related to salt and water stores, there is a minority of patients with hypertension associated with chronic renal failure who do not respond to dialysis-induced volume depletion with a lowering of the blood pressure. PRA in these patients tends to be higher than in the dialysis-responsive patients. Renin levels may be increased even before dialysis when (as a consequence of ECF volume expansion) renin levels would be expected to be lower than normal (273, 275, 277). In addition, renin levels may rise inappropriately in response to losses of sodium and water during dialysis, while the blood pressure may increase further. The response of high-renin patients with chronic renal failure to conventional antihypertensive drug treatment and dialysis may be inadequate, in which case drugs like propranolol that suppress renin secretion by the kidneys may be efficacious (286).

A certain number of patients on dialysis with refractory hypertension who had increased levels of circulating renin were subjected to bilateral nephrectomy to remove the source of renin (273, 277, 287–289). Following this procedure, plasma renin, aldosterone levels, and total peripheral vascular resistance decreased dramatically, and the hypertension became more readily controlled by dialysis and mild antihypertensive drugs. [In bilaterally nephrectomized patients, however, low levels of PRA may still be detected due to the small amounts of renin and/or renin-like enzymes produced by tissues other than the kidneys (290).] With the recent advent of $\beta$-adrenergic blocking drugs, which effectively lower renin secretion, and of very potent direct vasodilators such as minoxidil, it has been possible to normalize blood pressure in high-renin patients who are maintained on dialysis, thereby obviating the need for bilateral nephrectomy (286, 291, 292).

Two unusual renal causes of hypertension with high PRA may also be mentioned. One is hypertension associated with renin-producing tumors of the juxtaglomerular apparatus or renin-producing Wilm's tumors in children. The tumors produce an accelerated form of hypertension in young individuals, which is characterized by very high levels of PRA with secondary hyperaldosteronism, hypertensive retinopahy, and hypokalemic alkalosis. The abnormalities are totally reversed by excision of the tumor (293–295). Obstruction of the urinary tract by hydronephrosis can also cause increased renin secretion leading to severe hypertension, which is reversed when the obstruction is relieved (296).

## CONCLUSION

It is apparent from this selective review that derangements of the renin-angiotensin system and of sodium balance occur in a wide variety of clinical and experimental forms of hypertensive disease. Abnormal retention of sodium by the kidneys appears to be a hallmark of most forms of hypertension, although this is not always accompanied by increased secretion either of renin by the kidneys or of aldosterone by the adrenals. Both sodium retention (distending the vascular tree) and the level of peripheral arteriolar vasoconstriction (which is in part mediated by $A_{II}$) interact to maintain normal blood pressure in health and to produce and maintain an elevated arterial blood pressure in most primary and secondary hypertensive disorders. Abnormalities of the renin-angiotensin system and of sodium may also participate in the pathogenesis of selected cases of acute renal failure, although the precise interrelationships are not clear at this time. Current attempts to subdivide the very large population of patients with essential hypertension into high-, normal-, and low-renin subgroups are operationally useful in that they provoke controversy and further investigations into the pathogenetic mechanisms that lead to blood pressure elevation. Furthermore, indexes developed from measurements of renin, $A_{II}$, aldosterone, and sodium balance in these disorders may have prognostic implications and lead to more effective antihypertensive therapy.

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